Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA IN

COLTURE ARBOREE ED AGROSISTEMI
FORESTALI ORNAMENTALI E PAESAGGISTICI

Ciclo XXIII

Settore scientifico-disciplinare di afferenza: AGR/03

TITOLO TESI

MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF APPLE TREES UNDER PHOTOSELECTIVE COLORED NETS

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Esame finale anno 2011

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LIST OF ABBREVIATIONS

A_n	Net photosynthetic rate	µmol CO_2 m^{-2} s^{-1}
C_i	Intercellular carbon dioxide	µmol CO_2 mol^{-1}
E	Net transpiration rate	mmol H_2O m^{-2} s^{-1}
g_s	Stomatal conductance	mol m^{-2} s^{-1}
T_{leaf}	Leaf temperature	°C
$T_{leaf\text{-}air}$	Leaf-to-air temperature differences	°C
PPFD	Photosynthetic photon flux density	µmol m^{-2} s^{-1}
PAR	Photosynthetic active radiation	µmol m^{-2} s^{-1}
PSII	Photosystem II	--
NIR	Near infrared spectra	nm
TCSA	Trunk cross section area	cm^2
R:FR	Red : far-red light ratio	--
B:R	Blue : red light ratio	--
LMA	Leaf dry mass per area unit	mg cm^{-2}
$VPD_{leaf\text{-}air}$	Leaf-to-air vapour pressure differences	kPa
WUE	Water use efficiency	µmol CO_2 $mmol^{-1}$ H_2O
IWUE	Intrinsic water use efficiency	µmol CO_2 mol^{-1} H_2O
Φc	Phytochrome photoequilibrium	--
F_v'/F_m'	Efficiency of light harvesting by PSII	--
Φ_{PSII}	Quantum efficiency of PSII	--
ETR	Electron transport rate	µmol m^{-2} s^{-1}
Ψ_{leaf}	Leaf water potential	MPa
AGR	Absolute fruit growth rate	g day^{-1}
RGR	Relative fruit growth rate	mg g^{-1} min^{-1}
AER	Absolute shoot extension rate	cm day^{-1}

Chapter I

LITERATURE REVIEW

INTRODUCTION

Sunlight management in tree fruit orchards has been for several years an important research focus by pomologists and physiologists. Management of "light quantity", e.g. to maximize the amount of photosynthetic photon flux intercepted through cultural practices such as pruning, training system and tree arrangement has been a key factor to insure adequate fruit productivity and fruit quality in orchard systems (Jackson, 1980; Palmer, 1989; Corelli-Grappadelli, 2003). Effect of light quantity on key plant process such as leaf photosynthetic capacity, carbohydrate partitioning, vegetative and reproductive growth development have been widely studied in different fruit species such as peach (Nii & Kuroiwa, 1988) apple (Tustin *et al.*, 1992) and olive (Gregoriou *et al.*, 2007). However, the effect of "light quality" , which is the spectral composition of the sunlight, has been much less studied in fruit trees, due to the difficulty of conditioning the light environment of orchards (Baraldi *et al.*, 1998; Raparini *et al.*, 1999; Corelli-Grappadelli, 2003). Light quality composition changes widely into orchard canopies with important reduction of red (600-700 nm, R) and blue (400 – 500 nm, BL) light, while is observed an increases far-red (700-800 nm, FR) light component (Combes *et al.*, 2000; Awad *et al.,* 2001). Reduction of R proportion respect to FR (i.e. low R:FR ratio) induces inactivation of phytochrome activity with different morphological responses in woody plants associated to 'shade avoidance' phenomenon, such as increment on shoot elongation, apical dominance and reduced leaf thickness (Baraldi *et al.,* 1994; Gilbert *et al.,* 2001), while in peach trees it has been also demonstrated that prolonged irradiation with BL light induced shoot dwarfing , providing the evidence of action of specific blue light photoreceptor denominated cryptochrome which is responsible to induce an inhibitory effect on stem elongation in response to blue light (Rapparini *et al.*, 1999). However, most of these experiments have been carried out under controlled conditions and for our knowledge, the effect of light quality on plant response in fruit trees is still too limited under orchard environmental conditions (Baraldi *et al.*, 1998; Rapparini *et al.*, 1999). In the recent years and thanks to technological advances in supplemental lighting sources (e.g. light emitting diodes, LEDs) and photo-selective filters, light quality manipulation is an expanding technology and provide a more efficient and environmentally friendly alternative to morphologic, photosynthetic and yield plant performance control in greenhouse crops, such as ornamentals and vegetables (Hemming, 2009). This innovative concept has been recently introduced on horticultural and orchard systems production through develop of photo-selective netting approach. The approach combine physical protection of crops from abiotic and biotic stresses with selective light transmission in the R, BL, FR and NIR spectra of sunlight. However, probably physiological responses and/or mechanism behind of this technology are still unclear in fruit tree species due, in part, to limited information on this field research in fruit trees (Shahak *et al.,* 2008). The purpose of this article is to review the current status on light quality effect in fruit trees, with emphasis in physiological bases, plant responses and analysis of potential use of photo-selective netting for light quality management in fruit orchards.

LIGHT QUALITY COMPOSITION IN CANOPY TREES

Sunlight reaching the earth surface changes its spectral and energetic profile in part due to the normal climate variability and, in recent years, because of man-induced causes, such as the loss of stratospheric ozone that affects ultraviolet (UV) light absorption, or atmospheric pollutants and carbon dioxide (CO_2) levels that affects infrared (IR) light absorption (Grant, 1997). Thus, light spectrum composition that reaches the earth varies widely from the outer atmosphere to sea level, due to ozone effects that reduce the UV spectrum (below 400 nm) from 5% to 2%, and also because of carbon dioxide and water vapor that reduce the infrared spectrum (above 700 nm) of light from 67% to 53%. As a result of theses interactions, the visible spectrum (400 – 700 nm) is increased from 28% to 45% of the total light spectrum reaching sea level (Fig. 1-1) (Nobel, 1983). In general the radiation spectra that concerns plant physiologists is between 280 and 800 nm, which includes UV-B (280 - 320 nm), UV-A/B (300 - 400 nm), photosynthetically active radiation (PAR, 400 – 700 nm) and far-red (FR, 700 - 800 nm). The PAR radiation is subdivided into various bands and the most important for plant physiological processes are blue (BL, 400 – 500 nm), green (G, 500 – 600 nm) and red (R, 640 – 700 nm) light (Combes, 2000).

The spectral distribution of solar radiation also changes widely as the light penetrates and scatters within the plant canopy due to the structure and optical properties of the canopy components, such as leaves, fruits and branches (Grant, 1997). In general, the light environment inside the canopy is made up by two components: the unfiltered solar radiation (direct and diffuse), which has passed through gaps in the vegetation and the filtered radiation, which has been attenuated by the optical properties of reflectance and transmittance of the leaves (Smith, 1982).

It has shown that the optical properties of leaves play a crucial importance in the light spectrum modification at canopy level, with lower transmittance and reflectance in the UV, BL and R spectrum and higher in the G and FR and NIR light spectrum, while optical properties of reflectance and transmittance of leaves in woody plants can vary widely regard on the specie (Baldini *et al*., 1997). In walnut trees, values of leaf reflectance and transmittance in the FR spectrum are estimated to be near 50% and in the G spectrum around 20% for both optical properties (Combes, 2000), while in apple the reflectance and transmittance values in the G spectrum are 10% and 4%, respectively, and in the FR spectrum are near 50% and 30% (Fig. 1-2) (Palmer, 1977). In apple canopies, leaf optical properties can be widely modifying light composition at inner of tree which affects mainly the R:FR ratio (Awad *et al.* 2001). In this case R:FR ratio reached values of 1.6 at different outside positions of the tree canopy and near 0.5 in the inner canopy (Table 1-1). R: FR ratio also depending on seasonal canopy growth development. In peach trees, early in the season the R:FR values in the top and bottom of the tree canopy were around 1.1 in both parts, while later, near fruit harvest, they reached values of 0.5 at the top of canopy and 0.3 at bottom, almost a 50% of difference (Baraldi *et al.,* 1994).

PLANT RESPONSES TO LIGHT QUALITY

MORPHOGENESIS

The reduction of the R/FR ratio in the inner regions of the canopy may produce different morphological responses mediated by the photoreceptors called phytochrome (PHY) that are responsible for R and FR light signal transduction (Smith, 1982; Smith, 2000). PHY possesses the capacity of detecting wavelengths from 300 to 800 nm with maximum sensitivity in the R (600 to 700 nm) and FR (700 - 800 nm) wavelengths. Because of its spectral responses, PHY activity can be changing continuously through the two inter-convertible states that naturally occur: the R absorbing (P_r) and FR absorbing (P_{fr}) forms, which absorb maximally near 660 and 730 nm, respectively (Fig. 1-3) (Smith, 2000). Because the inner tree canopy has light rich in FR photons, the majority of the PHY pool is converted to the inactive P_r form, with loss of the active P_{fr} form. To estimate the PHY pool mediated responses, the R/FR ratio has been commonly used (Smith, 1982). However, most plant physiologists and photobiologists consider that the R/FR ratio does not accurately explain PHY-mediated plant responses and therefore other parameters have been proposed (Rajapakse & Kelly, 1994; Sager *et al.,* 1988). Mathematical models using spectral light information have been proposed to estimate the PHY photoequilibrium (Φc): the equilibrium state of biologically active P_{fr} form in relation to total phytochrome (P_{fr}/P_{total}). Φc provides a good indicator of expected photomorphogenic responses to a specific spectral light quality (Sager *et al*., 1988).

The curve that relates the R/FR ratio with Φc has been estimated in some orchard canopies such as walnut trees (Combes *et al.,* 2000) (Fig 1-3). It have shown a hyperbolic relationship between both components from the top to the inner canopy, with marked variations of R/FR ratio (from 0.3 – 1.2) and less important variations in Φc (from 0.35 – 0.68). Furthermore, estimated Φc is especially sensitive to R/FR changes in the range of 0.2 – 0.7 (shade conditions), but insensitive indeed to R/FR above about 1.0 (Combes *et al.,* 2000). Under high relative proportions of FR light the Φc is shifted toward the inactive P_r form. In these conditions, fruit trees exhibit different morphological changes, probably associated to "shade-avoidance" strategies evoked by decreasing P_{fr} form, such as shoot elongation, increased apical dominance and reduced leaf thickness (Baraldi *et al.,* 1994). Indeed for many trees the elongation rate of the shoots has an inverse relationship with the Φc (P_{fr}/P_{total})

(Gilbert *et al.,* 2001). In a classic study, Erez & Kadman-Zahavi (1972) demonstrated that apical growth activity of peach plants was strongly affected by changes in the Φc in P_{fr} form, but also demonstrated that BL light are quite important role in these responses. This observations were confirmed more later, when, still in peach, was demonstrated that prolonged irradiation with BL photons induced an inhibitory effect on shoot elongation, while BL alone had no effect on Φc, suggesting an independent action of blue light-dependent photoreceptor denominated crypthocrome (CRY). However, the morphological responses to BL light were widely modified and enhanced the inhibitory effect on stem elongation under lower level of Φc (i.e. lower activity of P_{fr}), providing therefore the evidence of the interaction of two (PHY and CRY) photoreceptors in the photocontrol of shoot growth in fruit trees (Baraldi *et al.,* 1998; Raparini *et al.,* 1999).

The effect of light quality on bud dormancy responds has been also indicated in fruit trees. Different physiological studies about the effect of photoperiod on growth and bud formation have pointed to an important role of the PHY system as the day-length sensor. Studies of woody plants with changed expression of PHY might suggest that perception of photoperiod is related to levels of PHY that apparently interact with biosynthesis of plant hormones, specially with gibberellin and abscisic acid during control of dormancy-related processes in fruit trees (Olsen, 2006). However, the role of PHY and photoperiod on dormancy release has not been totally understood. In general, it has been postulated that in a short day FR light is dominant, decreasing growth rate and stopping meristematic activity, while in a long day, R light is dominant and has the opposite effect (Erez & Kadman-Zhavi, 1972). Previous reports in peach demonstrated that limitation of illumination affected bursting of vegetative buds when it occurred shortly before sprouting. In general R light is more active on bud break and its effects on buds are reversed by a subsequent FR illumination, indicating a possibly role of PHY in this photoresponses (Erez *et al.,* 1968). However, the spectral light composition has no effect on flower bud burst or flower bud differentiation, as was demonstrated by Erez *et al.* (1966) and Baraldi *et al.* (1998). Probably the relationship between PHY system, daylength and bud growth and development presents complex interactions and depends also on genetic factors. In fact, Heide & Prestrud (2005) demonstrated that the photoperiod has no effect on growth cessation and dormancy induction in apple and pear plants, but a partial effect in *Prunus* species where a pronounced interaction of photoperiod and temperature exists on the regulation of growth cessation. This finding actually suggests that these species have a dual dormancy induction control system and one of them is mediated probably by light quality conditions (Heide, 2008).

Light quality conditions could be also involved in leaf anatomical and morphological differences between ‘sun’ and ‘shade’ leaves. In fruit trees such as peaches, apples and olives, the leaves that grow under shade conditions possess lower specific leaf area (mg cm^{-2}), larger leaf area and lower thickness compared to those growing under sunny conditions, due to anatomical changes at the palisade tissue level (Nii & Kuroiwa, 1988; Tustin *et al*., 1992; Gregoriou *et al.,* 2007). Although anatomical differences of sun and shade leaves can be attributed to light intensity changes, their developmental process and regulation are not well understood. In this sense it has been postulated a possible role of light quality and “shade-avoidance” mechanism regulated by PHY/CRY photoreceptors on morphological differences among sun and shade leaves (Kim *et al.,* 2005). Examination of peach leaf expansion showed that the combination of BL + FR light reduced significantly leaf area and the thickness of top and mesophyll palisade layers compared to R + FR light combination and neutral shade treatments. In addition, leaves exposed to R + FR light presented greater thickness of the palisade mesophyll in comparison with neutral shade (Fig. 1-5) (Baraldi *et al.,* 1998). The Φc values demonstrated that, opposite to the argument that indicates that low Φc generally increases leaf expansion, in this case leaf expansion was reduced under lower Φc (Table 1-2). This behaviour was confirmed in further experiments, concluding that probably the inhibitory effects of BL+FR light on leaf expansion and thickness are controlled by a specific BL receptor (CRY) independent of the PHY system (Rapparini *et al.,* 1999).

PHOTOSYNTHESIS AND LEAF GAS EXCHANGE

Different reports have shown the close relationship between light conditions and leaf photosynthetic performance. Peach and olive sun leaves, for example, were more efficient in CO_2 assimilation per unit chlorophyll compared to shade leaves. Furthermore, sun leaves presented greater starch accumulation in chloroplasts compared to shade leaves, indicating the greater photosynthetic capacity of those leaves (Kappel & Flore, 1983; Nii & Kuroiwa, 1988). The photosynthetic performance differences among sun and shade leaves have been attributed to morphological aspects. In general it has been postulated that leaves that grew under high light conditions are thicker, in order to arrange a greater number of chloroplasts along the palisade cell surfaces and for insuring a large investment in photosynthetic enzymes and adequate fluxes and fixation of CO_2 (Oguchi *et al.,* 2003). Some authors also demonstrated that spongy intercellular spaces were increased in shade leaves, proposing that such changes might alter CO_2 conductance from substomatal cavities to carboxylation sites in the chloroplasts, and restricting the photosynthetic rate in shade leaves (Gregoriou *et al*., 2007). It has been indicated that leaf age can strongly influence the photosynthetic acclimation, so when immature leaves are transferred from low light conditions to high light, leaf thickness and photosynthetic performance also increase, while mature leaves present little change in their thickness, which limits the photosynthetic capacity (Oguchi *et al*., 2003). However, in apple trees Mierowska *et al.* (2002) demonstrated that net photosynthetic rates were not affected by leaf age when leaves were exposed to direct sun by continuous pruning (young leaves) and later pruning (mature leaves), independent of the morphological traits, therefore a biochemical process can also be involved in the leaf photosynthetic acclimation to light environment conditions. In general, the biochemical mechanisms involved in photosynthetic acclimation are poorly understood, but the chlorophyll a/b ratio and light harvesting chlorophyll complex of photosystem II (LHC-II) has been described (Oguchi *et al.,* 2003). LHC-II contains between 32 and 42 molecules of chlorophyll per trimer (Chl a and Chl b) and 10 to 12 xanthophyll molecules (lutein, violaxanthin and neoxanthin) and constitute the basis for migration of excitation energy and for the dissipation of the excess light energy absorbed, protecting the photosynthetic apparatus from photoinhibitory damage (Van Amerongen & Van Grondelle, 2001). This aspect is quite interesting: in peach Losciale (2008) has demonstrated that beyond the saturation point net leaf photosynthesis is constant while photoinhibition (producing photodamage) increases, with negative consequences on the tree productivity. An effect of light quality on the LHCII complex has also been proposed, suggesting that light quality is an essential factor regulating the development of PSII (Xu *et al.,* 2004). In cotton seedling plants, small differences in FR/R ratio over variously coloured mulch surfaces have a significant effect on chlorophyll and LHC-II contents in leaves. Leaves of plants grown over white mulches had lower chlorophyll and LHC-II concentration than those grown over green, red, black and yellow surfaces (Bradburne *et al.,* 1989)

(Table 1-3). In strawberry, it was shown that chlorophyll content and the maximal photochemical efficiency of PS II were positively correlated with the R/BL light ratios, but the chlorophyll a/b ratio was negatively correlated with them, suggesting that PHY and/or BL light photoreceptor, probably CRY, can have an important role on leaf photosynthetic performance regulation (Xu *et al.,* 2004). In vitro experiments demonstrated that an increase in BL-enriched light fluence rate was more effective than R light in enhancement of the chlorophyll a/b ratio; therefore, a role was proposed for a Blue light photoreceptor (CRY) that detects low versus high fluence rates of light, and subsequently controls the light photosynthetic acclimation in leaves (Lopez-Juez & Hughes, 1995). These data were confirmed by current research that has shown that color of illumination during growth of pea plants affects LHC-II macro- aggregates (specially the a/b chlorophyll ratio), in intact leaves as well as isolated chloroplasts. The spectral conditions of all three types of illumination (R, BL and white light) used in this work do not allow converting the active PHY to the inactive form and vice versa. Therefore, the PHY most probably does not participate in the color effect on the LHCII macroaggregates, while a BL photoreceptor could be more involved by short and long- color memory mechanism (Gussakovsky *et al.,* 2007).

Light conditions also affects stomatal conductance which is closely linked to photosynthetic capacity in different fruit trees (Lakso; 1994; Flore *et al.,* 1985; Jifon & Syvertsen, 2003). Blue light has been mentioned as more effective than red light in causing stomatal opening or preventing stomatal closure (Farquhar & Sharkey, 1982). Blue light actives the plasma membrane H+ ATPase hyperpolarizing the membrane potential with simultaneous apoplast acidification and drives K+ uptake and accumulation in guard cells, increasing guard cell turgor and stomatal opening (Shimazaki *et al*., 2007). The stomatal response to blue light occur rapidly as was observed in vitro experiments (Fig. 1-6). In herbaceous plants the responses of stomatal opening and photosynthetic capacity to blue light has been widely documented (Taiz & Zeiger, 2009). However, to our knowledge it has not been studied in fruit trees.

GROWTH PARTITIONING

Light conditions also affect markedly the carbohydrate partitioning in fruit trees. Tustin *et al.*, (1992) and Corelli-Grappadelli *et al.* (1994) have underlined the essential role of light on carbohydrate partitioning patterns in apple, demonstrating that shoots exposed to full sunlight (9 – 17 leaves of development) are able to export photoassimilates 3 weeks after bloom, while similar export was reached by 70% shaded shoots only 5 weeks after bloom (13 to 22 leaves of development). These results were confirmed later when Bepete & Lakso (1998) demonstrated that under sunlight limitation the growing shoot tip has priority over the fruit for photo-assimilate allocation, especially early in the season when fruit number and cell division rates are establishing the potential of fruit development and load. Different photo-assimilate allocation is attributed to a “shade-avoidance” phenomen (Kasperbahuer, 2000). Kasperbahuer & Hamilton (1984) showed that long-term FR light exposure initiates events that result in relatively more photosynthates being partitioned to stems and less to leaves and roots as compared to plants that received R light, demonstrating furthermore that PHY manipulations influenced the amount of photosynthates present as starch grains in chloroplasts. In tomato, a higher proportion of FR spectrum allows to enhance of photosynthate allocation to developing fruits (Kasperbahuer & Hunt, 1998). In chrysanthemum shoots the translocation of photosynthates was affected by light quality. Reducing the FR spectrum reduced stem dry matter accumulation, but increased leaf dry matter accumulation (Rajapakse *et al.*, 1999). The possible role of PHY on carbohydrate partitioning in fruit trees has received special attention in recent research, where small changes in the FR/R ratio induced by kaolin particle film application could be influencing photoassimilate allocation to apple fruit, with important effects on improving fruit weight (Glenn & Puterka, 2007).

LIGHT QUALITY MANIPULATION BY PHOTOSELCTIVE NETTING PRINCIPLES AND HORTICULTURAL APLICATIONS

Manipulation of light quality and of the plant responses is an expanding technology and provide a more efficient and environmentally friendly alternative to morphologic, photosynthetic and yield in plant crops (Hemming, 2009). Recently a new approach has been developed based on plastic photo-selective colored nets with special optical properties (Shahak *et al.,* 2004). Depending on type of color, photo-selective nets absorb differentially the UV, BL, G, Yellow, R, FR and IR spectral regions (Fig. 1-7) and increase the ratio of scattered/diffused light, regulating photomorphogenetic/physiological plant responses (Shahak *et al.,* 2008). In kiwifruit, Blue nets reduced significantly the vigor of vines, whereas the Red and Grey nets appeared to stimulate vigor, while vine fertility was reduced under all colored nets (Basile *et al.,* 2008). In apple trees, Red/Black and Green/Black hail nets did increase significantly the number and length of bourse shoots, but reduced tree trunk diameter and return bloom. The effect of color of nets on tree growth was genetically influenced, with cv. “Fuji” more sensitive than “Pinova” (Solomakhin & Blanke, 2008). In the same species, coloured nets had an important effect on yield and fruit quality. Total fruit yield was significantly highest under Pearl net, as well the quantity of large fruits (>70 mm). Red and White nets were the second best for improving fruit size, while Black net reduced significantly the fruit size (Shahak *et al.,* 2008). Solomakhin & Blanke (2008) also reported increased apple fruit size under coloured hail nets, but without effect on yield. In peach, fruit grown under Red and Yellow nets were firmer, sweeter and fruit size was also improved (Shahak *et al.,* 2004). Photo-selective effects on fruit size and quality were also obtained in table grapes. Yellow net increased berry and cluster weight of “Superior” and “Red Globe” table grapes, while Red nets delayed the maturation of “Red Globe” compared with the White and un-netted control. Blue nets distinctly advanced maturation in “Superior” while delayed maturation in “Perlette” table grapes (Shahak *et al.,* 2008).

PHYSIOLOGICAL MECHANISMS

The most important effect of coloured nets is on vegetative and fruit growth development. The question is: Why does color of nets affect differentially these plant responses? Under Black nets the increase of vigour could be explained by positive effects of shading in the reduction of radiative and heat stresses. These would reduce plant water stress, increase photosynthesis and availability of carbohydrates and therefore increase tree vigour (Iglesias & Alegre, 2006). In South Africa, Smit (2007) found that leaves grown under Black shade net were 4.6 – 6.2°C cooler than controls in a hot day, while a significantly lower leaf to air vapour pressure deficit ($VPD_{leaf-air}$) was measured under netting, with increases in net CO_2 assimilation rate. In Israel, coloured shade nets reduced the maximum air temperatures by 1 - 5°C with 3-10% increase in the minimum daily relative humidity, but these parameters were not consistently affected by the coloured nets (Shahak *et al.,* 2004). Possible explanations have been attributed to “shade avoidance” responses mediated by PHY, but the R/FR ratio (principal component of PHY activity) does not change widely among color of nets (Solomakhin & Blanke, 2008). This aspect is important because changes in the pattern of carbohydrate partitioning have been attributed to PHY activity due to subtle changes in R/FR ratio (Kasperbahuer *et al.,* 2000). From the photomorphogenetic point of view, there is no consistent data yet demonstrating a possible role of PHY on the photo-selective effect of colored nets on plant growth. More clear differences have been found in the R/BL ratio among Blue and other net colors, a region of spectrum where a possible BL receptor (CRY) could be more implicated (Oren Shamir *et al*., 2001; Basile *et al.,* 2008).

On the other hand, changes in leaf function that determine the photosynthetic performance have also been proposed. Shahak *et al.* (2004) demonstrated that even though total PAR intensity was reduced about 30%, photosynthetic rates of apple exposed leaves were improved by the coloured nets, and the highest rates were obtained under Red nets. Changes in leaf chlorophyll content were detected under colored nets in citrus trees: plants under Blue nets had the greatest leaf chlorophyll a, b and total chlorophyll content, whereas leaves under Red nets had the lowest (Li & Syvertsen, 2006). Solomakhin & Blanke (2008) demonstrated that leaf chlorophyll synthesis under coloured hailnets was up to 46% under Red-Black and Green-Black nets in comparison with White nets, but this did not affect leaf photosynthesis. Although recent research demonstrated that color of illumination could be affecting the leaf chlorophyll content and maximal photochemical efficiency of PS II (Xu *et al.,* 2004; Gussakovsky *et al.,* 2007), this aspect is not yet confirmed under photo-selective coloured nets.

CONCLUSIONS

Light quality management in fruit trees promises a wide variety of applications. Plant key processes for fruit production such as vegetative growth, leaf photosynthetic capacity, and carbohydrate partitioning could be directly and indirectly linked to light quality signals mediated by PHY and/or CRY photoreceptors.

Photo-selective coloured nets are an innovative technological tool for light quality manipulation in fruit orchards. Depending in their light spectral quality, coloured nets might affect in different ways vegetative development, fruit growth and quality aspects in fruits. However, physiological mechanism involved in these photo-responses are not yet fully understood.

SUMMARY

Physiological bases and technological advances on light quality management in fruit trees were reviewed. Orchard canopies present marked changes in light quality, especially the red/far-red light ratio, which decreases almost by 50% from top to the inner canopy. A higher proportion of far-red light in these regions modifies the phytochrome (PHY) photo-equilibrium resulting in morphological changes associated to “shade avoidance syndrome”, such as shoot elongation and increased apical dominance. The influence of PHY on leaf palisade cell thickness in sun and shade leaves is not clearly demonstrated, while that a putative blue light receptor denominated crypthocrome (CRY) might be involved in leaf expansion and thickness. Color of light also affect chlorophyll content and function of photosystem II in leaves, while changes in the far-red/red light ratio could be influencing the photoassimilate allocation to fruit, a process mediated by PHY. Depending on light spectrum, photo-selective coloured nets influence different horticultural responses, however . physiological mechanisms of photo-selective nets are still unclear.

TABLES AND FIGURES

Table 1-1. Spectral composition of the sunlight at different canopy positions of "Jonagold" apple trees (Adapted from Awad *et al.,* 2001).

Position of tree	Spectral composition (% of total available light)					
	UV-A	Blue	Green	Red	Far-red	R/FR ratio
Above tree	6.1	8.7	17.7	20.4	10.7	1.9
Top	5.6	7.9	16.3	18.7	11.6	1.6
Inner	2.9	4.1	9.5	7.9	15.4	0.5
Outer east	5.2	7.4	16.0	19.0	11.7	1.6
Outer west	5.9	8.0	15.9	17.8	11.4	1.6
F-test	***	***	**	***	***	***

Table 1-2. Effect of different light spectrum conditions on leaf peach morphology (Source: Baraldi *et al.,* 1998).

Light treatment	Calculated PHY- Φc	Leaf area (cm^2)	Thickness (µm)		
			Top layer palisade	Palisade mesophyll	Spongy intercellular spaces (%)
Transparent	0.57	31.3 a	28.8 a	67.0 a	61 b
R/FR	0.49	33.2 a	23.5 b	46.2 b	59 b
BL/FR	0.13	24.3 b	20.9 c	38.5 c	67 a
Neutral	0.57	33.9 a	20.5 c	34.0 d	67 a

Table 1-3. Effect of different mulch surface on R, FR, FR/R ratio and relative light harvesting complex-II content in cotton leaves. (Source: Bradburne et al., 1989).

Mulch Surface Color	*Upwardly Reflected Light (%)*		*FR/R ratio*	Relative LHC-II Content (mg/g)
	R	FR		
Green	7.7	10.3	1.33	3.12
Black	5.2	5.7	1.09	2.85
Red	32.8	34.2	1.04	2.45
Yellow	44.3	44.7	1.01	2.30
White	43.3	43.5	1.00	2.10

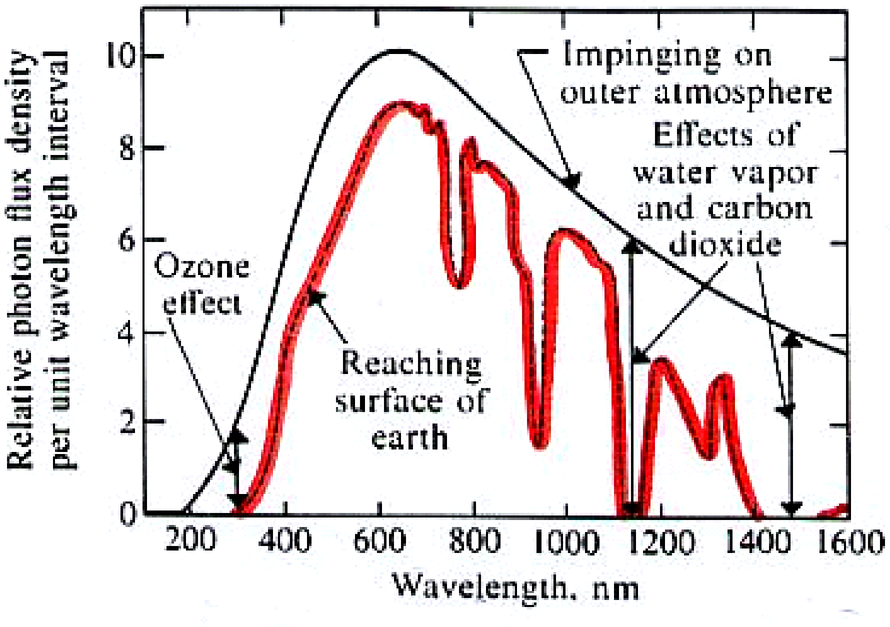

Fig. 1-1. Wavelength distribution of total sunlight at atmosphere and earth surface (Source: Nobel, 1983).

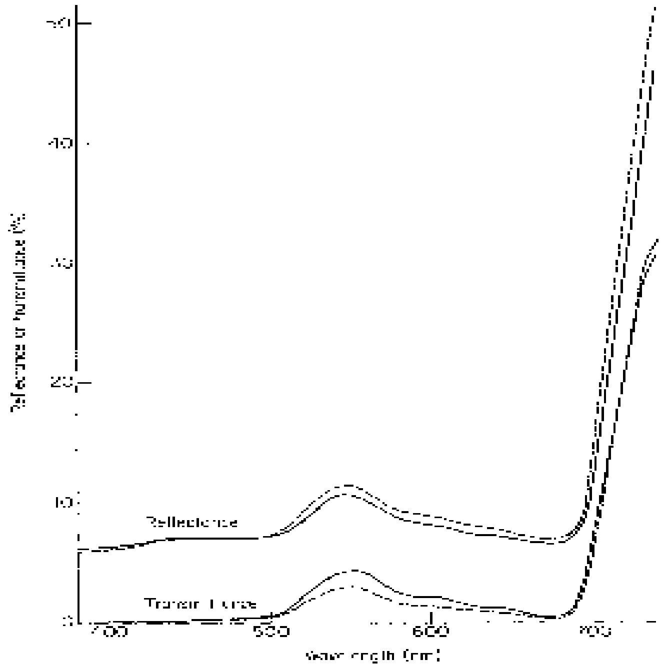

Fig. 1-2. Reflectance and transmittance pattern of apple leaves (Source: Palmer, 1977).

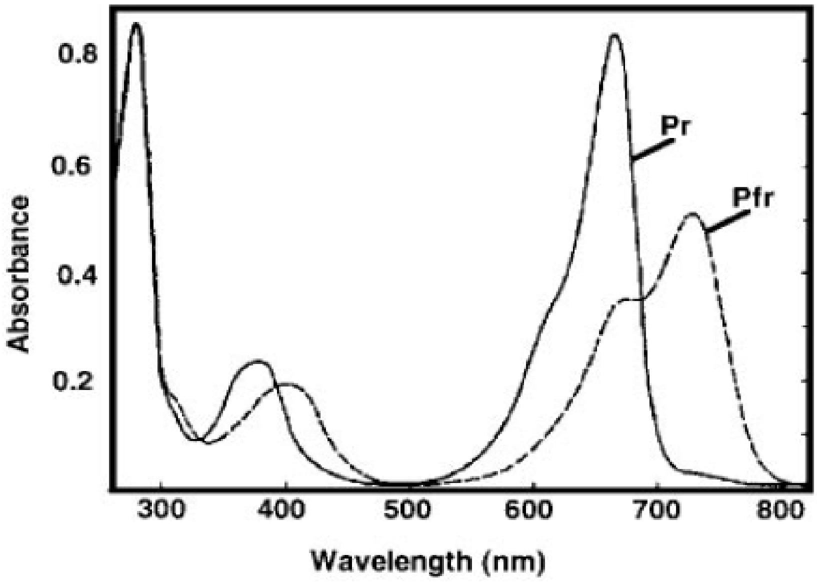

Fig. 1-3. Absorption spectra of P_r and P_{fr} phytochrome forms in plants (Adapted from Smith, 2000).

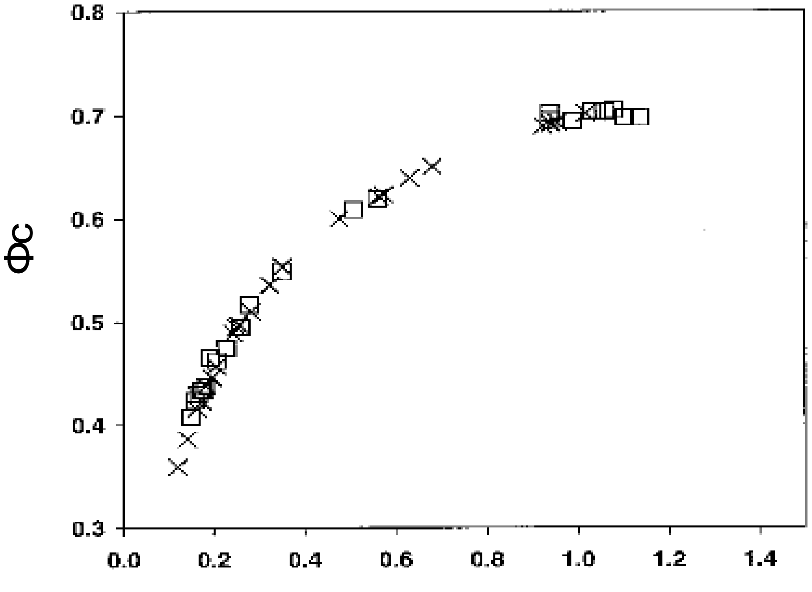

Fig. 1-4. Relationship between phytochrome photoequilibrium (Φc) and R/FR ratio on walnut canopy (Source: Combes *et al*., 2000).

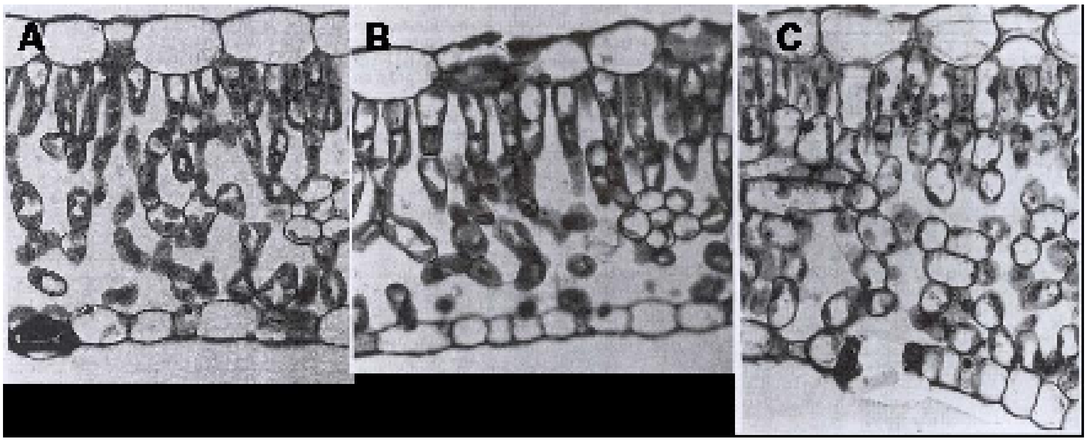

Fig. 1-5. Micrographs of peach leaf anatomy (palisade cells= PC and spongy intercellular space= SIS) grown under neutral (A), blue (B) and red (C) light (Source: Baraldi *et al.,* 1998).

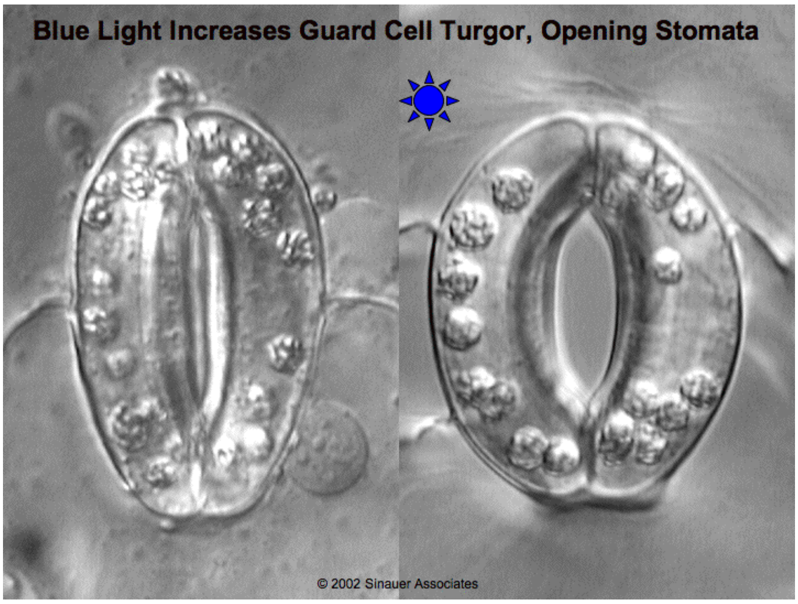

Fig. 1-6. Micrographs of influence of blue light on stomatal aperture in *Vicia Faba* (Source:Taiz & Zeigher, 2009).

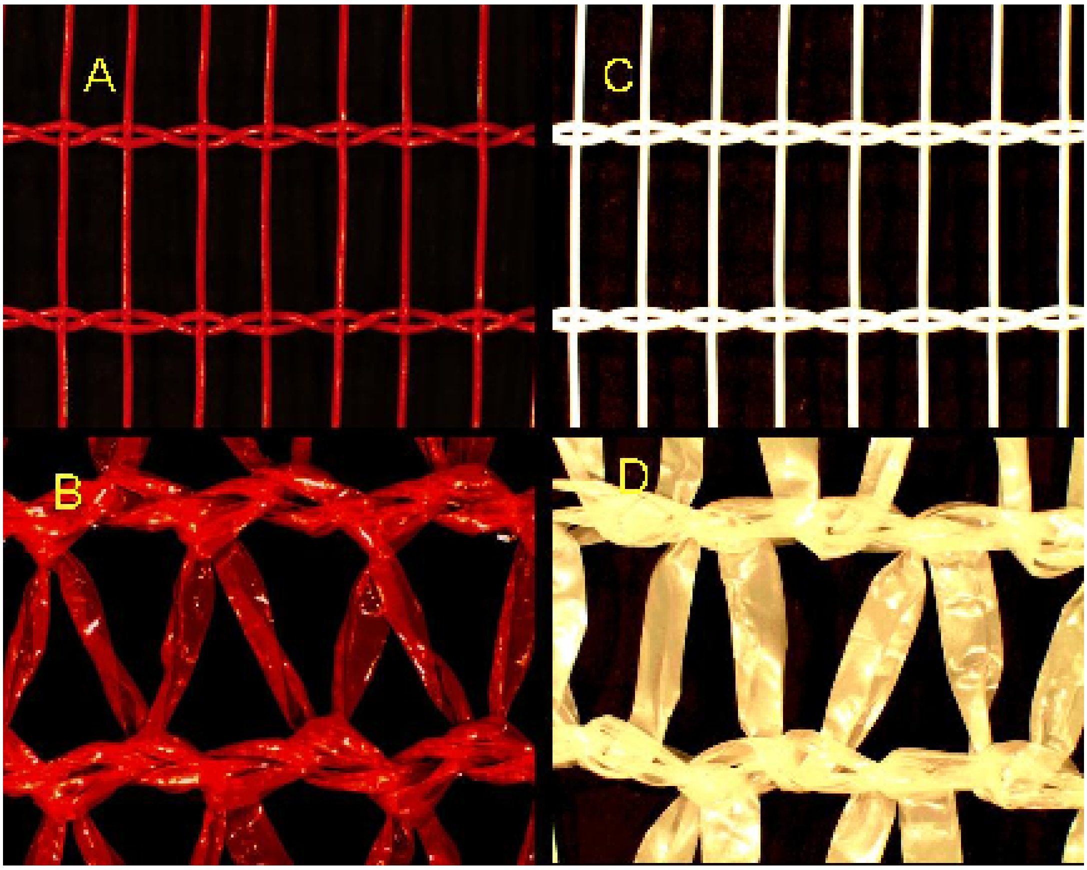

Fig. 1-7. Characteristics of red (A - B) and white (C - D) colored hail and shade nets.

Chapter II

LIGHT CONDITIONS UNDER COLORED SHADE NETS MODIFY GROWTH, LEAF MORPHOLOGY AND LEAF FUNCTION IN APPLE TREES

INTRODUCTION

Netting is an important practice in apple orchards to insure sustainable fruit production and quality. Apple orchards are covered with nets to protect fruit and plants from hail damage in areas with high frequency of hailstorms, as well from solar injury (sunburn) in regions with excessive solar radiation (Blanke, 2007; Gindaba & Wand, 2007a). Covering apple trees with nets causes multiple vegetative and reproductive plant responses, modifying tree vigour, fruit colour and sugar content, flower induction and consequently return bloom (Iglesias & Alegre, 2006; Leite *et al*., 2002). Most of these plant responses are related to changes in micro-environmental conditions such as air temperature and relative humidity, but mainly in light conditions (Solomakhin & Blanke*,* 2010). Light conditions play a fundamental role on different physiological responses in apple trees, including direct effects on leaf photosynthetic capacity (Wünsche *et al.,* 1996), carbohydrate partitioning (Tustin *et al.*, 1992; Corelli-Grappadelli *et al*., 1994), fruit colour (Ju *et al.,* 1999), flower induction (Lakso *et al.,* 1994) and changes in the growth pattern of leaves, shoots and fruits (Tustin *et al*., 1992; Bepete & Lakso, 1998). The recent introduction of field-durable, colored hail/shade nets demonstrates the interest in modifying the light quality conditions (spectral composition of sunlight) in the orchard, but physiological plant response to these particular light conditions in apple trees are still largely unclear (Shahak *et al.,* 2008), because most studies on light effects in apple trees have been focused towards understanding the effect of light quantity (i.e. the photosynthetic photon flux), but little information exists about the effect of light quality (Corelli-Grappadelli *et al.,* 2003).

In natural ambient, light quality changes widely into the canopy of fruit trees with important reduction of red and blue light and significant increment in far-red light (Combes *et al.,* 2000; Awad *et al.,* 2001). Under lower proportion red light respect to far-red light, trees exhibit different morphological responses associated to 'shade avoidance' strategies such shoot elongation, increased apical dominance and reduce leaf thickness which is mediated by phytochrome activity photoreceptor (Baraldi *et al.,* 1994; Gilbert *et al.,* 2001). Blue light also play an important role in these morphological responses in fruit trees regulated by a cryptochrome either independently or in conjunction with phytochrome (Rapparini *et al.* 1999). Continuous exposition to combined blue and far-red light induced the inhibition of shoot elongation, leaf thickness and apical dominance in peach trees (Erez & Kadman-Zahavi, 1972; Baraldi *et al.,* 1998). Moreover, experiments in herbaceous plant has demonstrated that blue and red light participate in the regulation of key leaf photosynthetic process such as stomatal aperture (Lin *et al.,* 2002) and the structural and functional characteristics of photosystem II in leaves grown to different light quantity regimens (Leong *et al.,* 1985; Lopez – Juez & Hughes, 1995; Matsuda *et al.,* 2004). Nonetheless, in orchard trees grown under filed conditions is complicate to ascribe morphological and physiological plant responses to specific light quality conditions, because changes in blue, red and far red light are also linked to changes in photosynthetically active radiation (Baraldi *et al.,* 1994).

This paper presents a study of the effect of colored shade nets on light quality and morpho-physiological responses in apple trees under field conditions. Moderate shading with colored nets probably modify morphological and functional traits in apple trees and leaves, induced mainly by changes in light quantity (PAR) as has been demonstrated in apple trees (Gindaba & Wand, 2007b; Smit *et al.,* 2008), but we postulate that light quality conditions under colored nets play also an important role in these plant responses, induced mainly by changes in the red, far-red and blue regions of the visible light spectrum. The aim of this study was to evaluate the effect of red, blue and neutral shade nets on following aspects: 1) changes in light quantity and quality; 2) tree growth and morphology; 3) leaf morphological and anatomical parameters and 4) leaf physiological parameters.

MATERIALS AND METHODS

Plant material and experimental design

The study was executed during 2009 year on two-year-old 'Fuji' apple maiden trees grafted on dwarfing M9 rootstock and formed by the 'knip-boom' nursery technique (Wilton, 2001). The trees were well provided with feathers. Previous to the start of the experiment (buds fully dormant), the weight, trunk diameter and height of ninety trees were measured and the trees were classified in two homogeneous groups by cluster analysis. Within each group, trees were placed in 40 l pots (1:2 sand and soil mix) and randomly assigned to three N-S oriented rows at 2.5 x 1.0 m spacing, to avoid mutual shading between rows. Water was supplied daily by a computer-controlled drip irrigation system. Every week (from full bud break until shoot growth ceased) 100 ml standard fertigation solution composed of nitrogen, potassium, phosphorus and microelements was applied to each tree. The trees were managed without pruning and all fruit were removed immediately after fruit set (May 2009).

Grey, Blue and Red colored shade nets (ChromatiNet®, Polysack Industries, Negev, Israel) with nominal shade factor 40% were studied. Due to frequent hail storms, it was not possible to use control plants exposed to full sunlight. A White, neutral net at 20% shading was consequently included as control. The light spectrum (400 – 800 nm) was tested on net samples at mid-day on a clear sunny day by a LI-1800 spectroradiometer (LI-COR, Lincoln, NE, USA). Spectra analysis (Fig.1) demonstrated that the Grey and White nets were wavelength neutral and reduced by the same amount full sunlight over the entire range (neutral nets). In contrast, the Red and Blue nets altered widely the spectral light distribution (photo-selective nets). Red net increased the light transmission in red and far-red spectra (600 – 800 nm), while the blue net enhanced the proportion of blue light (400 – 500 nm) and reduced the red light proportion (600 – 700 nm) (Fig. 1). Thus, White net was used as control: in comparison to the neutral Grey to examine the effect of light quantity and to the Red and Blue nets to evaluate the effect of changes of red and blue light proportion. The colored nets were placed from end of bloom (April 24, 2009) until leaf fall (end of November, 2009) on plots composed of 18 potted trees each. Nets were placed over a metal tunnel arc 6 m wide and 3.5 m high; the East and West sides of the tunnel were covered to the ground, while the North and South ends of the tunnel were left uncovered to insure good air circulation.

The experiment was arranged in a completely randomized design, selecting from the 8-12 central trees per color for all assessments; the rest of the trees server as guards to avoid the influence of unfiltered light from outside.

Light quantity and quality under field conditions

To validate the effect of colored nets under field conditions, light quantity and quality were reassessed under field conditions. Total photosynthetic photon flux density (PPFD, μmol m^{-2} s^{-1}) was measured by a QSO-S quantum sensor (Decagon Devices, Pullman, USA). Simultaneously, spectral light composition was quantified through an optical fiber (with a ping-pong ball acting as a diffuser fitted on the measuring head) which was connected to the LI-1800 spectroradiometer (LI-COR, Lincoln, NE, USA). Both sensors (quantum and optic fiber) were placed horizontally at 1 m above ground in the alleyway among the potted trees under full light conditions. Readings were replicated in four points each color net and three times during a sunny day (mid-morning, solar noon and mid-afternoon). Phytochrome and cryptochrome light-related parameters were quantified by red (600-700 nm)/far-red (700 – 800 nm) ratio (R:FR) and blue (400 – 500 nm)/red (600-700 nm) ratio (B:R) respectively, acoording to Kittas *et al.* (1999). The phytochrome photoequilibrium (Φc) was calculated by the model proposed by Sager *et al*., (1988).

Tree growth

Before leaf fall (November 2009), total shoot length and number of shoots were measured over a total of eight trees per color net. All apical and laterals shoots were classified according to three types of shoots (Costes *et al*., 2003): short (length ≤ 5 cm), medium (5 ≤ length ≤ 20 cm) and long (length > 20 cm). Before and after net deployment, trunk diameter (*d*) was measured 10 cm above the scion-rootstock union using a digital caliper and the trunk-cross section area (TCSA, cm^2) was calculated by the function $\pi (d/2)^2$. Return bloom was measured at full bloom the following season (April 21, 2010) by counting the total blooming clusters per tree, except those poorly developed which represented less than 3% of total clusters. Final values of return bloom were expressed as number of cluster per cm^2 of TCSA.

Leaf chlorophyll content, leaf area and leaf mass area ratio

In-vivo leaf chlorophyll content evolution was periodically measured, from 70 days after bud break (June 6, 2009) until shoot growth was stopped (September 26, 2009), using a SPAD 502 chlorophyll meter (Konica Minolta Sensing Inc., Japan). Chlorophyll measurements were made of forty-eight well-illuminated leaves per treatment, selected in the middle part of annual shoots. Additionally, when shoot growth stopped (September, 2009) forty mature and fully exposed leaves from the middle part of the shoots were randomly collected in each color net. Single leaf area (cm^2) was determined by an LI-3000 area meter (LI-COR, Lincoln, NE, USA) then immediately dried in a forced drought oven at 60° C and leaf dry mass per area unit (LMA, mg cm^{-2}) was estimated.

Leaf stomata density and length

When most leaves were fully expanded (July 29, 2009), twelve sun-exposed leaves per color net were randomly collected from the middle part of 1 year shoots. Leaves were wrapped in plastic bags placed in a cooler box with dry-ice and immediately transported to the laboratory for stomata analysis. Stomata density was determined by epidermal impressions with clear nail polish (Gitz & Baker, 2009). The leaf trychomes were removed by pressing an adhesive tape on each abaxial leaf surface. Then, leaves were painted with nail polish on two areas in the middle of the abaxial surface of each leaf, separated by the central vein. Once dried, the impression was gently removed and mounted on microscope glass slides with distilled water. Total stomata number was estimated in digital images, recorded on two square areas of 0.25 mm^2 per section and leaf by a CCD camera mounted on the light microscope at 40x magnification (Leitz DM RB, Germany).

Analysis of stomata length were obtained by scanning electron microscopy (SEM) using an adaptation of the methodology proposed by Baldini *et al.* (1997). Small pieces (~50 mm^2) samples obtained from four leaves per color net were excised from the middle of the abaxial surface of each leaf (previously peeled by adhesive tape), fixed in a formalin-free fixative (FineFix, Milstone, Bergamo, Italy), dehydrated in ethanol series and desiccated to critical point with a CPD 030 Critical Point Dryer (Balzers, Liechtenstein, Germany). Samples with the abaxial side up were mounted in aluminum SEM stubs with adhesive tape and then gold-coated with a SCD Sputter Coater (Balzers, Liechtenstein, Germany). Observations were made with a SEM 515 (Philips, Amsterdam, Netherlands) at 20 – 25 kV. Four photo-prints from different points for each sample and leaf of 503x magnification were taken, selecting a square area of 0.35 mm^2 for the measurements. Length of each stomata was measured as the distance between the outsides edges and then all stomata were classified in two categories: < 20 μm and > 20 μm.

Leaf mesophyll characteristics

Leaf mesophyll characteristics were analyzed by histological techniques (Baraldi *et al*., 1998). Samples of mature and well illuminated leaves (four leaves per color net) were collected from the middle part of one year shoots on July 20, 2009. Small pieces (~ 60 mm^2) were excised from the middle of the abaxial surface of each leaf, fixed in a formalin-free fixative (FineFix, Milstone, Bergamo, Italy), dehydrated in ethanol series (50, 70, 80, and 90%) and embedded in glycol methacrylate (Technovit 7100; Heraeus Kulzer GmbH, Werheim, Germany). Cross sections 3 μm thick were made using a rotary microtome (Reichert-Jung, Germany) and mounted on glass slides with distilled water. Twenty cross-sections were taken per each leaf sample. The quality of sections was continuously controlled by light microscope at 10x magnification and those with adequate morphological definition were then stained with toluidine blue for 5 min and photographed by a CCD camera mounted to the light microscope at 40x magnification (Leitz DM RB, Germany). Thickness (μm) of leaf blade, upper and lower epidermis, and palisade and spongy mesophyll were measured in at least four different section per each leaf and sample. Palisade to spongy mesophyll ratio was calculated (Baldini *et al.,* 1997). All microscopical measurements (stomata an mesophyll structure) were processed with Aequitas image analysis software program.

Leaf gas exchange

Net photosynthetic rate (A_n, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}) were measured using a LI-6400 gas infrared gas analyzer (LI-COR, Lincoln, NE, USA) at controlled and saturated photosynthetic photon flux (1200 µmol m^{-2} s^{-1} PPFD) provided by internal red/blue LED light. All measurements were made within the same period in which leaf samples for morphological analysis were taken, during three clear summer days (3, 8 and 13 July, 2009)I in the morning (9:30 – 11:00 h) and near solar noon (11:30 – 13:00 h), when maximum stomatal conductances are registered in apple trees (Giuliani *et al.,* 1997). Air temperature and VPD were ± 30.8 °C and ± 2.1 kPa in the morning and ± 32.5°C and 2.3 kPa near solar noon. Water use efficiency (WUE) as the ratio of A_n/E (µmol CO_2 $mmol^{-1}$ H_2O) and intrinsic water use efficiency (IWUE) as the ratio of A_n/g_s (µmol CO_2 mol^{-1} H_2O) were computed as proposed by Massonet *et al.* (2007).

Statistical analysis

Statistical tests were done by Statgraphics Centurion XVI (StatPoint, Inc., USA). Data were subjected to one- way ANOVA to test the significance ($p \leq 0.05$), followed by Student-Newman-Keuls test for multiple comparison of mean values.

RESULTS

Light conditions

On a sunny summer day, daily mean of light intensity (PAR) did not differ significantly among Red and Blue nets (Table 1). Light intensity under Red and Blue nets was 275 – 280 µmol PPFD m^{-2} s^{-1} lower than White (control) net and ca. 100 – 105 µmol PPFD m^{-2} s^{-1} higher than Grey net (Table 1). The R:FR value did not change significantly among White, Red and Grey nets and was up to 0.90, however this value was slightly reduced under Blue net (0.87), but with minimal impact on the phytochrome photoequilibrium (Φc) (Table 1). Blue net increased significantly the B:R ratio (0.92) compared to White (control) and Grey neutral nets (0.70), while Red net reduced largely this proportion (0.55) compared with other nets (Table 1).

Tree growth

Blue and Grey nets had a significant effect on the increment of total shoot length (4.5 – 5-4 m $tree^{-1}$) compared with White (3.2 m $tree^{-1}$) net, while total shoot length under Red was significantly lower (4.1 m $tree^{-1}$) than Blue net (5.4 m $tree^{-1}$) (Table 2). Blue net increased significantly the number of new shoots (33 shoots per tree) compared to White (control), Red, and Grey (22 – 25 shoots per tree) nets. Mean shoot length did not differ significantly among Red and Blue nets (±16 cm). However, mean shoot length measured under neutral Grey net was significantly higher (18.6 cm) than control White net (14.6 cm) (Table 2).

Distribution of shoots per class of length was also significantly affected by colored nets (Fig.2). The amount of short shoots (< 5 cm of length) was significantly higher under Blue net compared to other colored nets. Colored nets had no effect on the distribution of shoots in the medium category (≥ 5 length ≤ 20 cm), while Blue, Red and Grey nets increased the amount of long shoots (> 20 cm) compared to control

White net. More long shoots were found under Blue net compared to Red net (Fig. 2). Return bloom measured on April 2010 was increased up to 20% by Blue net (29 clusters cm^{-2} TCSA) compared with White (control) and other colored nets (Fig.3). Trees grown under Grey, Blue and Red nets presented slightly thinner (1.2 – 1.5 cm^2 of ΔTCSA) trunks, than those under control White net (~ 1.7 cm^2 of ΔTCSA), however there were no significant differences (Fig. 3).

Leaf chlorophyll content, leaf area and leaf mass area

Leaf chlorophyll content expressed as SPAD units differed significantly among colored nets, and the difference was marked during various moments in the growing season from 70 to 160 days after bud break (DABB) (Fig. 4). From 70 to 143 DABB, chlorophyll content of leaves that grew under Blue net was higher than those under Red, Grey and White (control) nets. During all season, leaf chlorophyll content under Blue net varies between 42 – 58 SPAD units, while under Red its range was between 39 – 55 SPAD units, equivalent to differences of 6% among both types of colored nets. Leaf chlorophyll content under Grey net was only slightly higher than other colored nets (Fig. 4).

Leaf morphology and anatomy

Single leaf area under Blue net was 3 – 4 cm^2 (18 – 19%) greater than control and other colored nets (Table 3). LMA also varied among colored nets (Table 3). LMA values of leaves grown under Blue, Red and Grey nets were 0.8 – 1.4 mg cm^{-2} (6 – 10 %) lower than control (White net). Furthermore, LMA in leaves grown under Red net was higher than under Blue net (Table 3).

Fig. 5 shows microscope images of cross-sections of leaf samples obtained from trees grown under the different colors. Leaves under White net were thicker (228.4 µm) than leaves grown under Red, Blue Grey nets (208 – 218 µm), but these differences were not significant (Table 3). However, palisade thickness was significantly affected by colored nets (Fig. 5; Table 3). Leaves that grew under White net presented three well defined layers of palisade cells compared to two well defined palisade layers observed in leaves from Red, Blue and Grey nets (Fig. 5). The palisade parenchyma of leaves under White (control) was 17% (116.2 µm) thicker than Red (96.2 µm), Blue (99.1 µm) and Grey (93.8 µm) nets. There were no significant differences in palisade thickness among Red, Blue and Grey nets (Table 3). The ratio palisade/spongy mesophyll was also significantly affected by colored nets. Leaves grown Grey, Red and Blue nets presented a reduction of 23% in the palisade/spongy mesophyll compared to control White net (Fig. 5 and Table 3). Leaf upper and lower epidermis thickness did not differ among colored nets.

Stomatal density was higher on leaves developed under White (512 stomata per mm^2) net than those developed under Red (423 stoma per mm^2), Blue (419.7 stoma per mm^2) and Grey (397 stoma per mm^2) nets (Table 3). On average leaves that grew under Red, Blue and Grey nets experimented a reduction of 20% in the number of stomata per mm^2 compared to control White net (Table 3). Regarding stomatal distribution per length, SEM analysis demonstrated that the frequency of stomata < 20 µm (i.e. smaller stomata) was greater under White net (control) than other colored nets. The proportion of stomata > 20 µm was not statistically affected by colored nets.

Leaf gas exchange

Evaluation of gas exchange during two moments of three summer days demonstrated that colored nets had a significant effect on leaf A_n, g_s, E, WUE and IWUE (Table 4). On average, during two moments of the day, g_s and E were incremented 38% and 29% by colored shade nets (Grey, Blue and Red), respectively, compared to White net (control). The effect of A_n changed depending on time of day. In the morning (9:30 – 11:00 h) A_n was increased 20% under colored nets (Grey, Blue and Red) compared to White net (control). Near solar noon (11:30 – 13:00 h), A_n under Blue net was 21% higher than control White net (Table 4), while A_n did not differ among Red, Grey and White nets. During two times of day, leaf IWUE under Grey, Blue and Red nets was on average 12% lower than control (White net). In the second moment of the day (11:30 – 13:00 h) WUE was reduced (7%) by Grey, Blue and Red nets compared with White net (Table 4).

DISCUSSION

The larger total length and amount of long shoots estimated under Blue, Red and Grey nets compared with White net (control), could be due to the considerable reduction of PAR intensity (c.a. 30% less than control) by colored shade nets. This is a typical shade-avoidance morphological response associated to the reduction of PAR intensity as described by Christophie *et al.* (2006). Our results confirm the findings by Smit (2007) and Solomakhin & Blanke (2008) of increased shoot length in different apple varieties under colored and neutral nets in comparison to full sunlight. However, we provide an additional finding: daily mean PAR intensity did not differ among Blue and Red nets, while under Grey net it was lower (Table 1);even so, the number of shoots formed under Blue net was higher than Red, Grey and White (control) nets (Table 2). The greater proliferation of shoots under Blue net is a morphological effect still unknown in apple trees. Experiments in peach trees suggested that other morphological responses induced by blue light can be altered by state of phytochrome (Rapparini *et al*., 1999), whereas studies in herbaceous plants have concluded that blue light promotes vegetative plant growth under natural ambient with reduced PAR availability and this process is regulated by phototropins (Takemiya *et al*., 2005).

Therefore, the effect of Blue net on shoot proliferation in apple trees suggests a response to shade avoidance and/or phototropism, where two or more photoreceptors are involved, linked to changes in light quality (more proportion of blue light) combined to light quantity (reduced PAR) as was observed in our study (Franklin, 2008). On the other hand, trees grown under Blue net presented more short shoots than all other nets, but also more long shoots than Red and White nets (Fig.2). Shoot growth is mediated by R:FR phytochrome and blue cryptochrome photoreceptors (Fankhauser & Chory, 1997). The R:FR ratio was significantly lower under Blue than other nets, which would indicate a probable phytochrome-mediated effect on shoot elongation that could explain the total shoot length increment by Blue net (Kasperbahuer, 2000; Smith, 2000). However, the less than 2% reduction of Φc, a more useful parameter to indicate the equilibrium state of biologically active phytochrome (Rajapakse & Kelly, 1994), by Blue was probably not enough to justify an effect on morphological responses mediated by phytochrome (Combes *et al.*, 2000). The blue light proportion in relation to red light (B:R ratio) under Blue net, on the other hand, was incremented 37% in relation to neutral Grey and White (control) nets, and over 65% in relation to Red net. A higher proportion of blue in relation to red light induces shoot dwarfing via reduction of stem elongation, a process mediated by the blue light cryptochrome photoreceptor, which was confirmed by the greater amount of short shoots observed under Blue net (Baraldi *et al.*, 1998; Rapparini *et al.*, 1999; Cummings *et al.*, 2008).

Larger PAR reduction and increases of vegetative growth under netting have been closely related to poor flower induction and decreases of return bloom during the next year in apple trees (Leite *et al.,* 2002; Solomakhin & Blanke, 2008). However, in the present study we did not observe any reduction in return bloom by Blue, Red and Grey nets respect to White net (control) (Fig. 3). These results are similar to those reported by Smit (2007), who demonstrated that shade netting did not negatively influence flower induction in apple trees. This could be due to the fact that in our study all fruit were removed in the previous season, allowing sufficient carbohydrate build-up for adequate flower induction (Wünsche & Ferguson, 2005). Interestingly, return bloom was 20% greater under Blue net. Rossi *et al.* (1990) and Baraldi *et al.* (1994) showed in apple and peach that flower bud differentiation can be modulated by R:FR ratio, but they did not observe the participation of blue light to this process. In this study, leaf photosynthesis under Blue showed a tendency (non significant) to be slightly higher than other colored nets (Table 5), and this could explain in part the more abundant return bloom observed at the start of the 2010 season: the enhanced carbohydrate availability might have increased flower induction (Lakso, 1994; Corelli-Grappadelli, 2003). Although trunk cross sectional area did not differ among colored nets, tree trunks showed a trend to be thicker under White net (control) than Red, Blue and Grey. This trend was also observed by Solomakhin & Blanke (2008) in different varieties of apple under colored hail nets. Given the large differences in shoot growth observed among shade nets respect to control White net, these results suggest that light conditions under colored nets could be affecting the apple sink – source relationships between organs within-tree scale as has been proposed in other plant crops (Kasperbauer & Hunt, 1998).

Leaves grown under Blue, Red and Grey net exhibited a lower LMA compared with White (control) net. Reduction of LMA is a common responses of apple leaf to reduction in the light availability (Corelli-Grappadelli, 2003). These results confirm previous reports of a large reduction of LMA by shading in species such as peach (Kappel & Flore, 1983), hazelnut (Hampson *et al.,* 1996), olive (Gregoriou *et al.,* 2007) and apple (Tustin *et al*., 1992). LMA differences result from changes in leaf structure thickness, leaf area and amount of photosynthetic products and components induced mainly by light conditions (Gregoriou *et al*., 2007). Thus, sun leaves grown under higher irradiance present smaller area, greater thickness, more palisade layers, higher a/b chlorophyll ratio, more soluble carbohydrates and a large investment in photosynthetic components: chloroplasts, nitrogen and enzymes; in comparison with shade leaves (Lichtenthaler *et al*., 1981). Microscopy analysis showed that our leaves grown under Red, Blue and Grey nets presented one layer of palisade cells less than control (White net); whereas palisade thickness and palisade/spongy mesophyll thickness under colored shade nets were reduced 17% and 23%, respectively, compared to control (Fig.4 and Table 4). These results indicate that the reduction of LMA by color shade nets was due mainly to reduced palisade thickness and increases in the fraction of mesophyll air space, which are common adaptive responses of shade leaves to improve light absorption by chloroplasts (Smith, 1997). They also confirm the plasticity of the mesophyll structure in apple leaves to changes in light intensity under netting (Solomakhin & Blanke, 2010). Although the Red net presented a slightly lower palisade/spongy mesophyll ratio than Blue and Grey nets, there were no significant differences in mesophyll structure among leaves grown under these nets. These results indicate that, under the conditions of our experiment, PAR availability was more relevant than light quality to the modulation of the anatomical differentiation of palisade cell development as was also suggested by Kim *et al.* (2005), but in opposition to studies which observed that reduced palisade cell enlargement in shade peach leaves grown in the inner tree canopy is regulated by changes in light quality (Baraldi *et al*., 1994). The changes observed in stomatal density confirm these results. Leaf stomata density did not differ among colored Red, Blue and Grey nets, but on average presented a reduction of 20% in the stomata density compared to control (White net), similarly to Nii & Kuroiwa (1988) and Gregoriou *et al.* (2007), who found a lower stomatal density in peach and olive leaves exposed to neutral shading. Nevertheless, the differences in LMA among Red and Blue nets can not be explained by quantitative changes in light availability because the leaves grown under Red and Blue nets received the same quantity of incident PAR (Table 1). Moreover, under Blue net leaf expansion and total chlorophyll content were widely increased compared with other shade and White (control) nets (Table 3). The differences in leaf expansion explain the differences in LMA among Blue and Red nets (Gregoriou *et al.,* 2007). The increase of leaf chlorophyll content by Blue net and/or the reduction of leaf chlorophyll content by Red nets has also been reported in citrus and ornamental plants (Li & Syvertsen, 2006; Stamps & Chandler, 2008). Although greater leaf area and chlorophyll content is another common response of fruit tree leaves to reduced irradiance (Kappel & Flore, 1983; Nii & Kuroiwa, 1988; Solomakhin & Blanke, 2008), we demonstrate that, regardless PAR intensity, chlorophyll content and leaf expansion are leaf characteristics stimulated by light quality composition and specifically by Blue net. Recent studies have underlined the importance of blue light on leaf photosynthetic acclimation to different light environments; they suggested that responses such as leaf expansion and chlorophyll enhancement are stimulated by blue light in environments with reduced PAR and/or Red light to improve the photosynthetic performance. It is a process regulated by specific photoreceptors denominated phototropins (Matsuda *et al.,* 2004; Takemiya *et al.,* 2005).

On the other hand, if we consider the differences of LMA, chlorophyll content and leaf expansion among Red and Blue nets, these suggest that apple leaves grown under Blue nets resemble ‘shade leaves’, whereas those grown under Red net resemble “sun leaves” (Lichtenthaler *et al.,* 1981). However, leaf photosynthetic capacity expressed as A_n (µmol CO_2 m^{-2} s^{-1}) measured at the same PAR (1200 µmol PPFD) did not differ among Red, Blue and neutral Grey nets (Table 5). In fact, Blue net presented a A_n

value slightly more higher than Red nets and other colored nets. These results demonstrate that even though leaves under Blue net resemble morphologically "shade leaves", this trait had no influence on its photosynthetic performance, so more complex mechanisms must be involved in this response (Matsuda *et al.,* 2004; Takemiya *et al.,* 2005).

Colored shade nets (Grey, Red and Blue) increased significantly g_s respect to control (White net). These results seem contradictory, because in leaves under White net we observed higher stomatal density, normally associated to higher g_s (Gregoriou *et al.*, 2007). However, we have to point out that in fruit trees, including apple, g_s strongly depends on environmental factors (T_a, VPD and PPFD) and internal plant conditions (water status, tree growth and mineral nutrition) (Warrit *et al.*, 1980; Jones, 1998; Giuliani *et al.,* 1997). The environmental T_a, VPD and PPFD, as well as irrigation and fertilization practices were the same in our experiment. One possible explanation is due to morphological differences of tree growth that were induced by colored nets. Recent studies demonstrated that irrespective of water status, reduced shoot growth by dwarfing rootstocks affected negatively g_s in apples trees, while rootstocks promoting rapid shoot growth were linked with high g_s (Atkinson *et al.*, 2000). Based on these reports, higher g_s in leaves grown under Blue, Red and Grey nets can be linked to higher shoot growth expressed by these trees compared to control (White net). One further explanation can be related to the stomatal regulatory function of photosynthesis (Jones, 1998). Even though higher g_s was related to increases in A_n and *E* under Red, Blue and Grey nets, at solar noon (Table 5) A_n did not differ significantly among Red, Grey and White (control) nets but was higher under Blue. These results indicate the presence of additional non-stomatal factors regulating A_n under colored nets, probably associated to structural and biochemical properties of the photosynthetic apparatus (Lichtenthaler *et al.,* 1981). The greater palisade thickness is involved in higher Rubisco activity and CO_2 fixation (Oguchi *et al.,* 2003). From this point of view, leaves grown under lower light availability (Red, Blue and Grey) nets were more limited by photosynthetic machinery (lower palisade cell development), while those leaves grown under White net (control) did not present similar limitations in their photosynthetic machinery (greater palisade thickness), therefore they must have been under prevailing stomatal limitation (i.e. reduced stomatal conductance as observed in our study) (Farquhar & Sharkey, 1982). This hypothesis was confirmed by the differences in WUE and IWUE observed. Leaves under Grey, Blue and Red nets presented lower WUE and IWUE compared to control (White net), so in this sense leaves grown under White net are more water conserving, but the A_n is more limited by stomatal conductance (Massonet *et al.,* 2007). However, the present analysis can not fully explain the factors regulating gs and A_n differences under colored nets. For example, it has been observed in apple and other fruit trees that continuous exposure to moderate shade netting increases the g_s, E and A_n by light and thermal stress reduction (Smit, 2007; Smit *et al.,* 2008; Jifon & Syverstsen, 2003). In this study, we did not evaluate this component, but the differences in light intensity and light quality exhibited by colored nets could also be playing an important role on 'in situ' micro-environmental regulation of leaf stomatal conductance and A_n and the other gas exchange parameters studied here.

CONCLUSIONS

Irrespective of PAR intensity, Red and Blue shade nets affected differently key morphological parameters in trees (number of shoots, shoot length and bloom return) and leaves (leaf expansion, chlorophyll content and LMA), governed mainly by changes in red and blue light proportions.

However, an effect of light quantity (PAR) was also observed and more clearly in anatomical changes (palisade thickness and stomata density) in apple leaves.

Colored netting altered leaf gas exchange parameters: leaves grown under colored shade nets presented higher A_n and E but lower IWUE and WUE compared to control (White net) and closely related to changes in stomatal conductance and mesophyll structure. However, this study did not allow us to identify with accuracy the effect of light quality and main factors involved in these differences. Further research is necessary in this perspective.

These findings may provide a new perspective on shade netting, confirming the potential of Blue and Red shade nets for modulating some of the physiological and agronomical traits in apple orchards.

TABLES AND FIGURES

Table 2-1. Total photosynthetic photon flux density (PPFD), red:far-red (R:FR), blue:red (B:R) ratios and phytochrome photoequilibrium (Φc) estimated under different colored shade nets.

Net treatments	PPFD (μmol m^{-2} s^{-1})	Wavelength ratios R:FR	B:R	Φc
White (control)	1020.0 ± 13.5[1]	0.960 ± 0.006	0.700 ± 0.006	0.704 ± 0.0002
Red	745.2 ± 11.6	0.930 ± 0.004	0.550 ± 0.007	0.700 ± 0.0008
Blue	736.9 ± 15.7	0.870 ± 0.004	0.920 ± 0.010	0.690 ± 0.0006
Grey	639.5 ± 7.6	0.940 ± 0.010	0.720 ± 0.006	0.702 ± 0.0010

[1] Each value represents the mean ± SE of four readings taken during the morning, solar noon and afternoon. R:FR estimated as (600-700 nm)/(700-800nm), B:R estimated as (400 – 500nm)/(600 – 700 nm).

Table 2-2. The effect of colored shade nets on total shoot length, total shoot number and mean shoot length in 'Fuji' apple trees.

Net treatments	Total shoot length (m $tree^{-1}$)	Total shoot number (n° $tree^{-1}$)	Mean shoot length (cm)
White (control)	3.2 c[1]	22.2 b	14.6 b
Red	4.1 bc	25.3 b	16.4 ab
Blue	5.4 a	33.3 a	16.6 ab
Grey	4.5 b	24.3 b	18.6 a

[1] Mean separation within columns by the Student-Newman-Keuls test; n= 8, $p \leq 0.05$.

Table 2-3. The effect of colored shade nets on morphological and anatomical characteristic in 'Fuji' apple leaves.

Net treatments	Leaf expansion (cm^2)	LMA (mg cm^{-2})	Leaf tissues thickness (µm)				Palisade/Spongy mesophyll ratio	Stomata density (n° mm^{-2})	Stomatal distribution per length category (n° mm^{-2})	
			Total	Upper epidermis	Lower epidermis	Palisade			< 20 µm	> 20 µm
White (control)	19.4 b [1]	12.8 a	228.4	13.7	9.6	116.2 a	1.35 a	531.8 a	266.0 a	243.5
Red	20.6 b	12.3 b	218.9	13.4	9.7	96.2 b	0.9 b	442.2 b	101.8 b	288.7
Blue	24.9 a	11.8 c	215.2	13.6	9.8	99.1 b	1.12 b	438.7 b	149.5 ab	298.4
Grey	22.6 ab	10.8 d	208.4	13.3	9.9	93.8 b	1.07 b	420.9 b	229.6 ab	256.5

[1] Mean separation within columns by the Student-Newman-Keuls test; n= 40 leaves (for leaf area and LMA) or 12 leaves (for stomatal density), or 4 leaves (for leaf tissue thickness and stomatal distribution), $p \leq 0.05$.

Table 2-4. The effect of colored nets on gas exchange parameters measured in 'Fuji' apple leaves during two periods in the day. Leaf measurements were repeated during three days (3, 8 and 13 July, 2009) and averaged.

Parameters/period	Colored nets			
	White (control)	Red	Blue	Grey
9:30 – 11:00 h				
A_n (µmol CO_2 m^{-2} s^{-1})	12.7b[1]	14.8a	15.8a	15.2a
g_s (mol m^{-2} s^{-1})	0.15b	0.21a	0.22a	0.21a
E (mmol H_2O m^{-2} s^{-1})	3.5b	4.7a	4.7a	4.6a
WUE (µmol CO_2 $mmol^{-1}$ H_2O)	3.6	3.2	3.4	3.3
IWUE (µmol CO_2 mol^{-1} H_2O)	81.6a	68.4b	73.0b	71.0b
11:30 – 13:00 h				
A_n (µmol CO_2 m^{-2} s^{-1})	11.7b	12.5ab	14.2a	13.5ab
g_s (mol m^{-2} s^{-1})	0.17b	0.22a	0.23a	0.24a
E (mmol H_2O m^{-2} s^{-1})	4.2b	5.1a	5.2a	5.4a
WUE (µmol CO_2 $mmol^{-1}$ H_2O)	2.8a	2.5b	2.7ab	2.6b
IWUE (µmol CO_2 mol^{-1} H_2O)	68.6a	60.0b	62.3b	58.6b

[1] Mean separation within rows by the Student-Newman-Keuls test; n= 6, $p \leq 0.05$.

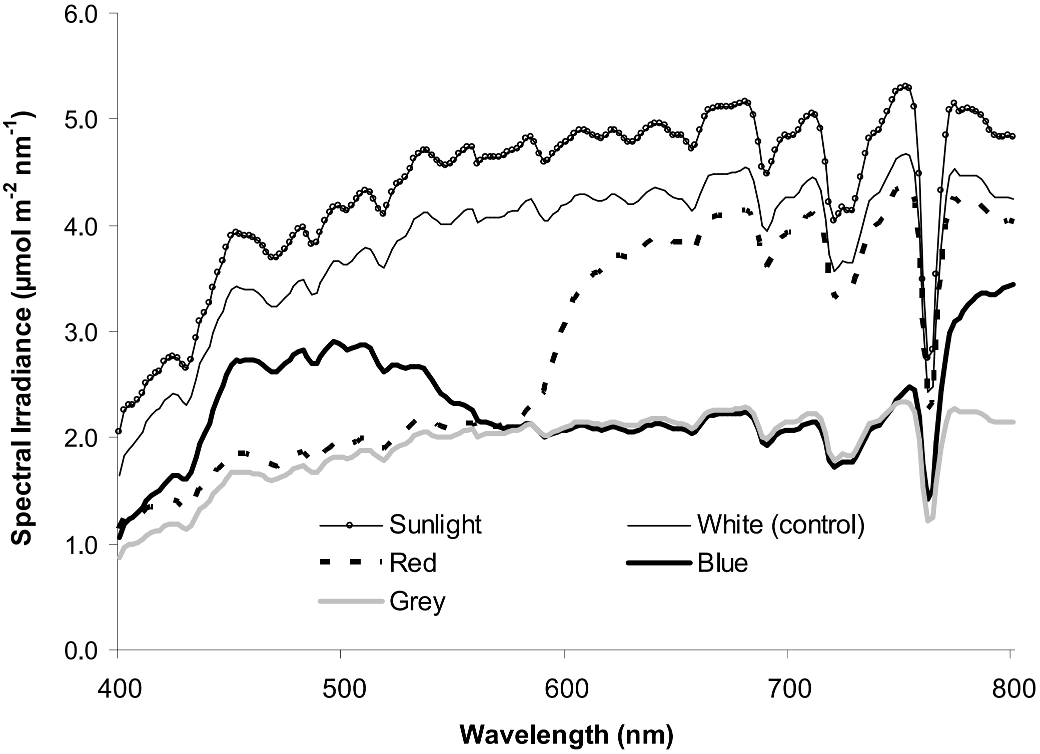

Fig. 2-1. Spectral distribution of light for different colored shade nets respect to full sunlight.

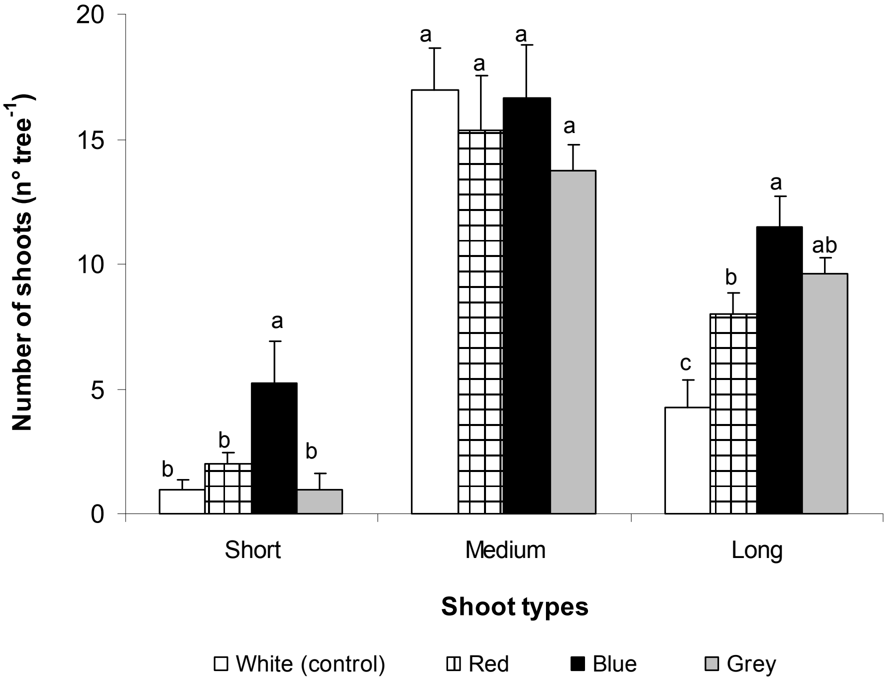

Fig. 2-2. The effect of colored shade nets on short (< 5 cm), medium (5 – 20 cm) and long (>20 cm) shoot development in 'Fuji' apple trees. Each value represents the mean ± SE of 8 trees. Mean separation between treatments by the Student-Newman-Keuls test. ($p \leq 0.05$).

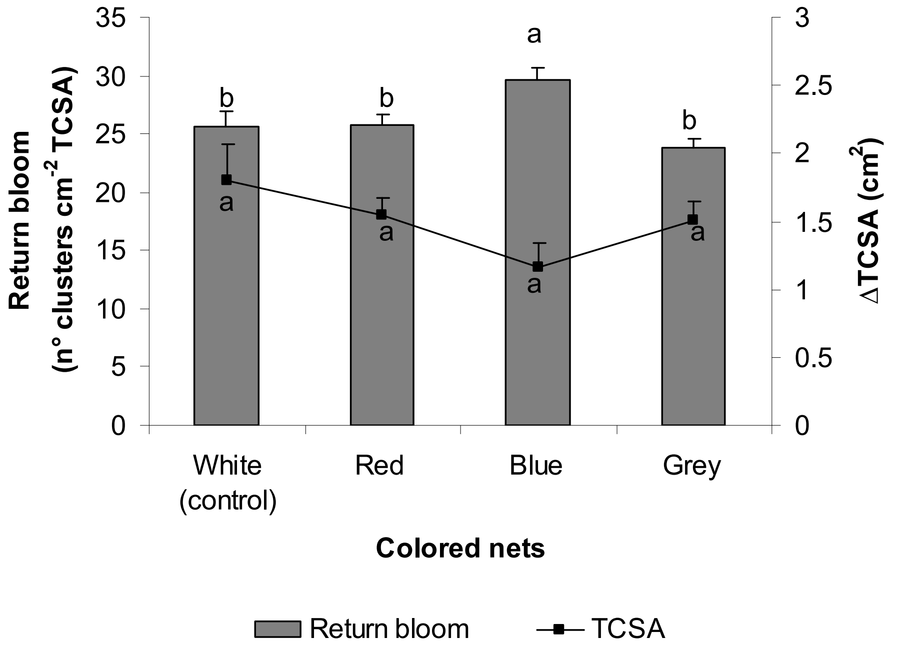

Fig. 2-3. The effect of colored shade nets on return bloom and trunk cross section area increment (ΔTCSA) in 'Fuji' apple trees. Each value represents the mean ± SE of 8 trees. Mean separation between treatments by the Student-Newman-Keuls test. ($p \leq 0.05$).

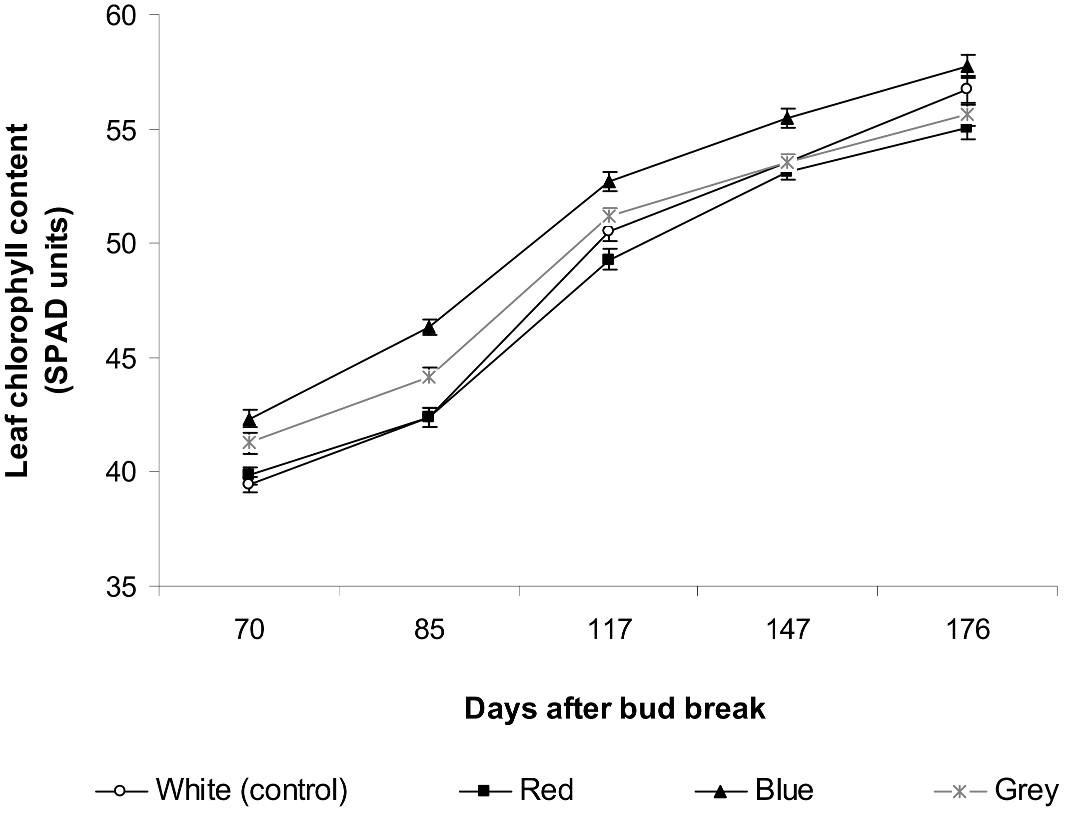

Fig. 2-4. The effect of colored shade nets on chlorophyll content evolution in 'Fuji' apple leaves. Each value represents the mean ± SE of 48 leaves.

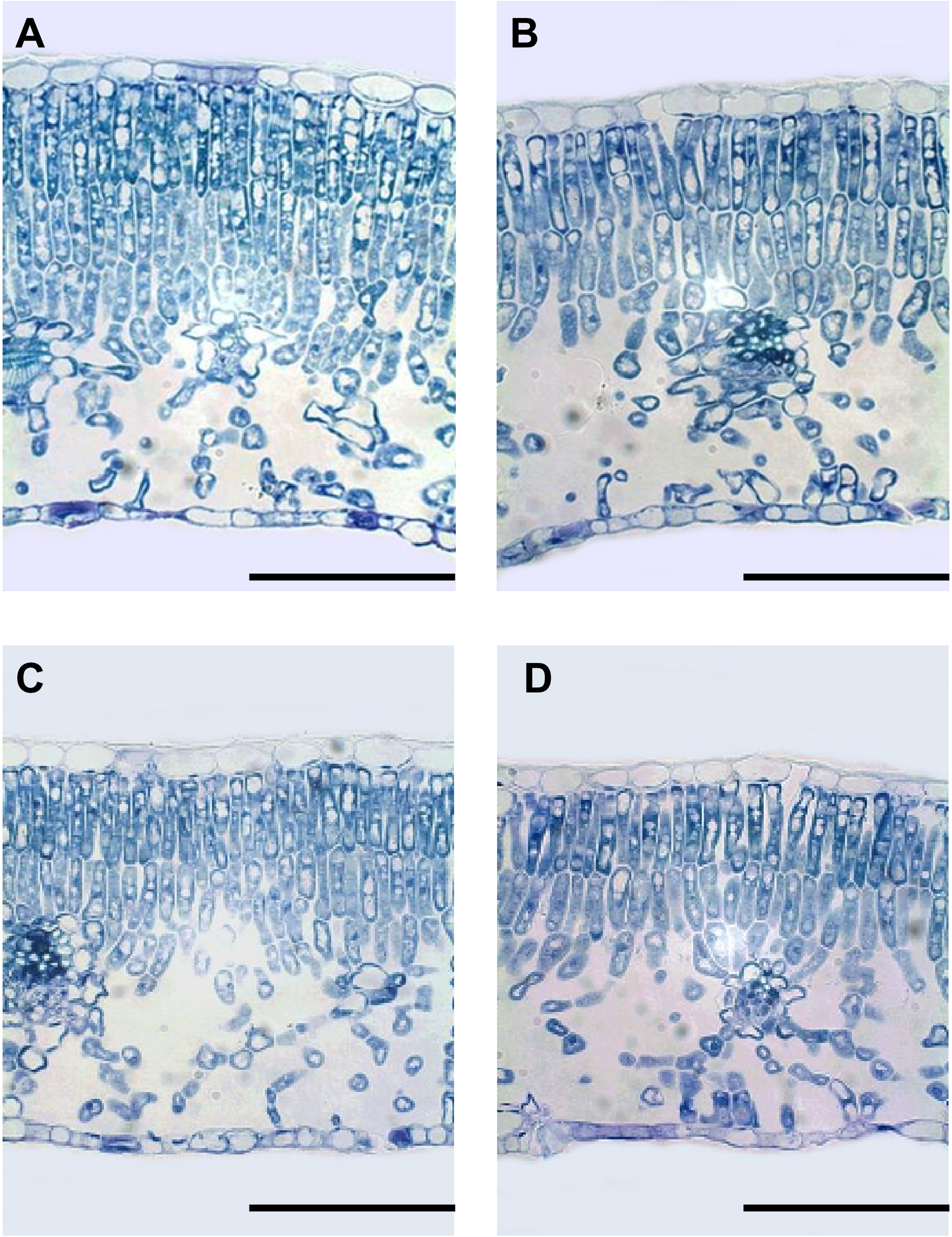

Fig. 2-5. Micrograph of leaf cross-section of 'Fuji' mature apple leaves grown under White (control) (A), Red (B), Blue (C) and Grey (D) nets. Magnification 40x. *Bars* = 100 µm.

Chapter III

REGULATION OF STOMATAL CONDUCTANCE AND PHOTOSYNTHETIC CAPACITY IN APPLE LEAVES BY COLORED SHADE NETTING

INTRODUCTION

The use of shade or hail netting over orchards to reduce plant stress produced by hailstorms and/or excessive radiation is a cultural practice that is rapidly expanding in the main apple production areas of the world (Vittone *et al.,* 2006; Blanke, 2007; Yuri *et al.,* 2000). Netting considerably alters micro-environmental conditions in apple orchards, such as reduction of air temperature, increase in relative humidity and overall reducing incoming solar radiation (Iglesias & Alegre, 2006; Solomakhin & Blanke, 2010). Shade netting leads to reductions in availability of photosynthetic photon flux (PPF), as well reduced leaf temperature and leaf-to-air vapour pressure deficit ($VPD_{leaf\text{-}air}$) which are strongly linked to variations in leaf stomatal conductance (g_s) and net CO_2 assimilation rate (A_n) (Jifon & Syvertsen, 2003). In apple trees the effect of shade and/or hail netting on leaf g_s and A_n has been reported, but with contradictory findings depending on climate conditions and cultivars: Under cloudy climate conditions reduction of 10 – 20% PPF by black hail nets was associated with significant reduction in leaf A_n in 'Fuji' and 'Jonagold' apple trees (Solomakhin & Blanke, 2008; Widmer, 1997). Similarly, under sunny and hot climate conditions, Gindaba & Awad (2007) reported a down-regulation of photosynthetic capacity and decreased g_s in 'Royal Gala' apple leaves by 35% of PPF reduction under black shade nets. However, Ebert & Cassiera (2000) reported a larger leaf A_n in apple trees grown under black hail nets. Furthermore, installation of 20% black shade nets in 'Fuji', 'Braeburn', 'Royal Gala' and 'Cripps Pink' did not reduce leaf A_n and in some instances was beneficial, while leaf g_s in most cases was higher under netting, specially in days with more extreme temperature and radiation conditions (Smit *et al.,* 2008).

In fruit trees increased leaf A_n under netting has been shown to be closely linked to reduced midday leaf g_s depression by mitigation of daily extreme values in $VPD_{leaf\text{-}air}$ as was observed in citrus trees (Jifon & Syvertsen, 2003; Raveh *et al.,* 2003). This interpretation is also applicable to apple trees, since apple leaf A_n is closely and positively related to changes in leaf g_s (Lakso, 1994) and beyond a certain threshold apple leaf g_s decreases linearly as $VPD_{leaf\text{-}air}$ increases (Flore *et al.,* 1985; Giuliani *et al.,* 1997; Massonet *et al.,* 2007). Improving leaf A_n capacity by netting could also be linked to non-stomatal mechanisms, as was observed in citrus trees, where shade nets reduced photo-inhibition by excessive light and consequently improved the photochemical efficiency of photosystem II (Medina *et al.,* 2002). However research advances in this direction are still limited in other fruit species such as apple trees (Corelli-Grappadelli & Lakso, 2007). Moreover, although netting can significantly alter leaf g_s and A_n capacity in apple trees, all studies documented above have been carried out with neutral nets and therefore effects are primarily due to reductions in light quantity (Smit *et al.,* 2008), but without reports about the effect of light quality conditions (i.e. spectra composition of light).

In recent years and thanks to technological advances in supplemental lighting sources (e.g. light emitting diodes, LEDs) and photo-selective filters, light quality manipulation (specially blue, red and near-infrared light) have demonstrated positive effects on gas exchange and photosynthetic performance in ornamentals and vegetable crops (Hemming, 2009). Manipulation of red and blue light differentially affects the regulation of leaf stomatal aperture (Shimazaki *et al.,* 2007), structural and functional characteristics of photosystem II (Matsuda *et al.,* 2004) and leaf mesophyll CO_2 conductance (Loreto *et al.,* 2009), while near-infrared screens reduced heat stress and increases photosynthetic capacity in greenhouse crops (Hemming, 2006). This innovative concept has recently been introduced in orchard systems through development of colored nets (Shahak et al., 2004; Solomakhin & Blanke, 2010). Colored nets provide selective light transmission in the red, blue and near-infrared regions of sunlight, however the effect of this technology on leaf g_s and A_n capacity in apple trees are not completed understood (Shahak *et al.,* 2008). This work tests the hypothesis that, although shade netting (via a reduction of light quantity) could be increasing apple leaf g_s and A_n, these responses can be altered by color net filters (modification of light quality) as well. To test this hypothesis changes in leaf g_s and A_n capacity in apple trees grown under colored shade nets with differential light transmission in the red, blue and near-infrared spectra were evaluated. Micro-environmental analysis (light, temperature, water status) as well gas exchange and fluorescence measurements were carried out to study the effect of colored shade nets on leaf g_s and A_n potential in apple trees.

MATERIALS AND METHODS

Plant material and experimental design

The research was carried out during 2010 at the University of Bologna Experiment Research Station (44°30’N; 10°36’E; 27 m a.s.l.). A ‘mini’ orchard was designed using three-year-old ‘Fuji’ apple trees grafted on dwarfing M9 rootstock, planted in 40 l pots (1:2 sand and soil mix) and randomly assigned to three N-S oriented rows at 2.5 x 1.0 m spacing. When flowering and pollination were considered completed (April 29, 2010) Grey, Blue and Red colored shade nets (ChromatiNet®, Polysack Industries, Negev, Israel) with nominal 40% shade factor were placed on plots of 18 trees. White neutral net at 20% shading was included as control, because the occurrence of hail storms did not allow to use plants completely exposed to full sunlight. Spectral (400 – 1100 nm) light transmission of all the nets was tested by a LI-1800 spectroradiometer (LI-COR, Lincoln, NE, USA) (Fig. 3-1). The Grey and White nets were wavelength neutral and reduced by the same amount sunlight over the entire range. The Red net reduced light transmission in the blue and green range (400 – 580 nm) and increased it in the red and near-infrared spectrum (600 – 1100 nm). The Blue net enhanced the proportion of blue and green light (400 – 580 nm), reduced the red (600 – 700 nm) and increased the light transmission in part of far-red and near-infrared spectra (750 – 1100 nm) (Fig. 3-1). Nets were placed over a metal tunnel arc 6 m wide and 3.5 m high; the East and West sides of the tunnel were covered to the ground, while the North and South ends of the tunnel were left uncovered to insure good air circulation. Each plot was separated from the others by at least 10 – 12 m of uncovered terrain, to avoid the influence of scattered light within adjacent color nets.

Tree crop load was adjusted to 5 fruits/cm^2 of trunk cross section area (~ 20 fruits/tree) (Robinson, 2008) by hand thinning performed between 19 – 25 days after full bloom (May 10 - 16, 2010). Water was supplied daily by a computer-controlled drip irrigation system. Every week (from full bud break until shoot growth ceased) 100 ml standard fertigation solution composed of nitrogen, potassium, phosphorus and microelements was applied to each tree. The experiment was arranged in a completely randomized design, with tree guards on all sides of the 4 – 8 central trees per color net, which were used for all assessments; the precaution served to avoid the influence of unfiltered light from outside.

Spectral irradiance under field conditions

Spectral irradiance, expressed as, μmol m^{-2} s^{-1} nm^{-1}, was measured under field conditions during three times of the day (3 hours of solar noon, solar noon and 3 hours after solar noon) after canopy completion, to ensure that the test trees were indeed subjected to uniform light conditions. Measurements were taken between 400 – 1100 nm (Visible plus NIR spectra) using an optical fiber (with a ping-pong ball acting as a diffuser fitted on the measuring head) which was connected to the LI-1800 spectroradiometer (LI-COR, Lincoln, NE, USA).

Diurnal leaf gas exchange measurements

Daily course of net photosynthetic rate (A_n, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), intercellular carbon dioxide concentration (C_i, μmol mol^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), leaf temperature ($T_{leaf,}$ °C), leaf-to-air vapour pressure differences ($VPD_{leaf\text{-}air}$, kPa) and photosynthetic photon flux density (PPFD, μmol m^{-2} s^{-1}) were measured using a LI-6400 infrared gas analyzer (LI-COR, Lincoln, NE, USA) under ambient conditions. All measurements were made on six mature and fully exposed leaves selected from the middle part of annual vegetative shoots and on three central trees (two leaves per tree). Measurements were carried out at 1 hour intervals, from 8:30 a.m. to 16:15 p.m. and repeated during two clear and hot summer days (representative of conditions at the experiment site) with similar daily mean value of PAR radiation and air temperature: 8 July, 2010 (1026.2 ± 272.8 PPFD and 34.5 ± 2.5 °C) and 9 July, 2010 (1057.0 ± 226.7 PPFD and 35.2 ± 2.8 °C). Water use efficiency (WUE) as the ratio of A_n/E (μmol CO_2 $mmol^{-1}$ H_2O) and intrinsic water use efficiency (IWUE) as the ratio of A_n/g_s (μmol CO_2 mol^{-1} H_2O) were computed as proposed by Massonet *et al.* (2007).

Leaf photosynthesis and chlorophyll fluorescence measurements

Net photosynthetic rate (A_n, µmol CO$_2$ m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), leaf to air vapour pressure deficit (VPD $_{\text{leaf-air}}$, kPa) intercellular CO$_2$ concentration (C$_i$, µmol mol^{-1}) and chlorophyll fluorescence parameters in light-adapted leaves were measured using an open infrared gas analyzer system (LI-6400, LI-COR, Lincoln, NE, USA) equipped with a leaf chamber fluorometer head (6400-40 LCF, LI-COR, Lincoln, NE, USA). Measurements were made during two moments: mid-morning (9:30 – 11:00 h.) when g$_s$ in apple leaves normally reaches the maximum peak and at midday (11:45 – 13:20 h) a time when leaf g$_s$ shows a marked depletion (Cheng & Luo, 1997). All measurements were made on six mature and sun-exposed leaves selected from middle part of vegetative shoots and repeated during four summer days (23, 25, 28 and 29 June, 2010). Light was provided by internal red/blue LED sources and light intensity was set as the average of photosynthetic photon flux density (PPFD) measured under colored nets during the two moments during the day. Thus, measurements were made at 1050 and 1450 µmol m^{-2} s^{-1} PPFD in mid-morning and midday, respectively.

The following fluorescence parameters for light-adapted leaves were calculated using the equations proposed by Genty *et al.* (1989) and reviewed by Maxwell & Johnson (2000):

Efficiency of light harvesting by PSII open reaction centers was calculated as:

$$F_v'/F_m' = (F_m' - F_o') / F_m' \qquad (1)$$

where F$_m$' is the maximum fluorescence signal after a saturating flash of light and F$_o$' is the minimum fluorescence was measured after a dark pulse.

The fraction of absorbed photons by PSII that are used for photochemistry (i.e. quantum efficiency of PSII) in light-adapted leaves was calculated as:

$$\Phi_{PSII} = (F_m' - F_s) / F_m' \quad (2)$$

where F_s is the steady-state value of fluorescence. After that, linear electron transport rate (ETR) referred to actual flux of photon (µmol m^{-2} s^{-1}) driving PSII was calculated as:

$$ETR = \Phi_{PSII} \times f \times PPFD \times \alpha \quad (3)$$

where f is fraction of absorbed quanta used by PSII, which is assumed to be 0.5 for C_3 plants (Earl & Ennahli, 2004), PPFD is the quantity of photon flux density (µmol m^{-2} s^{-1}) and α is the leaf absorbance, which in mature apple leaves is 0.89 (Palmer *et al.,* 1977).

Finally, photochemical quenching was estimated as:

$$qP = (F_m' - F_s) / (F_m' - F_o') \quad (4)$$

Leaf characteristics

Well after shoot growth stopped (October 5, 2010), in-vivo leaf chlorophyll content was measured by a SPAD 502 chlorophyll meter (Konica Minolta Sensing Inc., Japan) on twenty leaves per treatment. Simultaneously, forty leaves per treatment were randomly collected, scanned and the area of each leaf was measured by Aequitas image analysis software (DDL Ltd, Cambridge, UK). Then, leaves were dried in a forced draught oven at 60° C for the determination of leaf dry mass per area unit (LMA, mg cm^{-2}). All parameters were estimated on fully expanded and well illuminated leaves and from the same positions as those used for gas exchange analysis (middle part of vegetative shoots).

Tree and soil water status

Predawn and daily course (4:00 a.m. – 22:00 p.m.) of leaf water potential (Ψ_{leaf}, MPa) were measured with a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR) during a partially sunny day (August 17, 2010). All measurements were made on five sun exposed and fully expanded leaves from the middle part of annual vegetative shoots. Simultaneously, water evaporation from the pots was estimated using samples of 1:2 sand:soil mix, which were placed in plastic glasses of 160 cm^3 of capacity. Samples of sand:soil mix were taken at field capacity and then the plastic glasses were placed at fully exposed positions under each color net. The soil water evaporation rate from the soil:sand mix was estimated on ten plastic glasses for each treatment and at the same times of measurement of leaf water potentials, weighing each plastic glass and expressed as mg H_2O evaporated cm^{-2} h^{-1}.

Statistical analysis

The effect of net treatments were analyzed by one- way ANOVA to test the significance at $p<0.05$ and 0.01, respectively, followed by Student-Newman-Keuls test for multiple mean value comparisons. Regression analysis was used to estimate the relationships between variables. Statistical tests and fitting parameters of regression models were done by Statgraphics Centurion XVI software (StatPoint Technologies, Inc., Warrenton-Virginia, USA).

RESULTS

Spectral Irradiance

Fig. 3-2 shows spectral irradiance under colored shade nets measured during a clear summer day. All colored shade nets reduced significantly the amount of radiation transmitted in the visible spectrum (400 – 700 nm) compared to control (White net), with more reduction under Grey net (Fig. 3-2). Between 440 and 520 nm (blue light) transmission under the Blue net was 0.2 – 0.5 $\mu mol\ m^{-2}\ s^{-1}\ nm^{-1}$ greater than under the Red net. However, from 590 to 890 nm (red light and part of NIR) radiation transmission was significantly lower under the Blue compared to the Red net (Fig. 3-2). Maximum differences in radiation transmission between the Red and Blue nets were observed in the red region (600 – 700 nm), where Blue net reduced up to 1 – 1.3 µmol m^{-2} s^{-1} nm^{-1} compared to Red net (Fig. 3-2). In the NIR region (700 – 1100 nm) the radiation transmission under Red net was similar to control White net, while under Grey net it was the lowest compared to control and all colored shade nets (Fig. 3-2). The Blue net reduced the amount of radiation transmitted only in part of the NIR spectrum (700- 890 nm) compared to Red and White nets.

Daily course of leaf gas exchange under ambient light conditions

The daily course of ambient photosynthetic photon flux density (PPFD), leaf temperature (T_{leaf}) and leaf-to-air vapour pressure deficit ($VPD_{leaf\text{-}air}$) measured under colored nets is shown in Fig. 3-3. Red and Blue nets reduced PPFD in the same proportion compared to White net, whereas under Grey net daily PPFD was the lowest (Fig. 3-3 A). At midday, PPFD under Blue and Red nets was about 1100 µmol m^{-2} s^{-1} and under Grey net 930 µmol m^{-2} s^{-1}, representing a reduction of 26% and 37% of PPFD compared to White net (1490 µmol m^{-2} s^{-1}). Daily mean PPFD did not differ significantly among Red and Blue nets, while under Grey net it was the lowest ($p<0.001$, Table 3-1). Reduction of radiation load by colored shade nets was accompanied by a reduction in T_{leaf} and $VPD_{leaf\text{-}air}$ (Fig. 3-3 B and C). Maximum T_{leaf} differences were reached between 11:30 – 15:00 h. Although daily mean air temperature (T_{air}) did not differ among colored nets ($p>0.05$), daily leaf-to-air temperature differences ($T_{leaf\text{-}air}$) under Grey and Blue nets were about 0.5 °C lower than control (White net) (Table 3-1). $VPD_{leaf\text{-}air}$ under Grey and Blue nets were 0.3 – 0.4 kPa lower than control (White net) and the difference was more marked at midday (12:50 – 14:00 h), whereas leaves under Red net presented similar values of $VPD_{leaf\text{-}air}$

compared to control (White net) during the day (Fig. 3-3C). Reduced $VPD_{leaf-air}$ values under Blue and Grey nets resulted in significant increases of stomatal conductance (g_s) (Figure 3-4A). From 10:30 to 14:00 h leaf g_s under Blue and Grey nets exceeded by 30 – 50 mmol m^{-2} s^{-1} that measured in leaves grown under Red and White nets. Daily mean leaf g_s under Blue net was the highest ($p<0.05$) (190 mmol m^{-2} s^{-1}) and 27 % greater than those obtained under Red and White control nets (150 mmol m^{-2} s^{-1}) (Table 3-1). However, daily mean leaf g_s under Grey net did not differ from the Red and White control nets (Table 3-1). Early morning (8:30 h) leaf g_s was poorly related to $VPD_{leaf-air}$ changes (Fig. 3-5A) and the relationship was not significant for any net treatment ($p>0.05$; Table 3-2). However, after mid-morning (10:30 h) and when $VPD_{leaf-air}$ values exceeded 2.0 - 2.5 kPa a linear negative relationship was found between g_s and $VPD_{leaf-air}$ (Fig. 3-5B), which was closer still at midday (12:50h) (Fig. 3-5C). In both periods the relation was statistically significant ($p<0.05$; Table 3-2). At midday, a tighter relationship between leaf g_s and $VPD_{leaf-air}$ was found under Red and White nets (R^2 = 0.86 and 0.89, $p<0.001$; respectively) compared to Blue (R^2= 0.71) and Grey net (R^2= 0.57), while in both periods the lowest significance was found under Grey net (R^2= 0.41 and 0.57; $p<0.05$ and 0.01, respectively).

Daily mean leaf A_n did not differ among net treatments ($p>0.05$; Table 3-1). However, daily leaf A_n pattern was similar to g_s and leaf A_n under Blue and Grey nets tended to be higher than under Red and White nets (Fig. 3-4 B). For all net treatments, values of g_s and A_n peaked between 9:30 and 10:30 and then they declined during the rest of the day (Fig. 3-4 A and B). A linear positive relationship was found between leaf A_n and g_s in the early morning, mid-morning and midday readings (Fig. 3-6; Table 3-3). In the early morning and at mid-morning the relationship was less strong under Blue net compared to other colored nets and control (R^2= 0.53 and 0.67, respectively; $p<0.01$ Table 3-3). However, at midday the weakest relationship was found under control (R^2= 0.44, $p<0.05$) and the highest under Red and Blue nets (R^2= 0.91 and 0.81, respectively; $p<0.001$) (Table 3-3).

Daily transpiration rate (E) and intercellular carbon dioxide concentration (C_i) also differed among colored nets (Fig. 3-7 A and B). Maximum values of leaf E under Blue and Grey nets (6.1 and 5.8 mmol H_2O m^{-2} s^{-1}, respectively) were reached at midday (12:50 h) and then gradually declined during the afternoon. However, under Red and White control nets, maximum values of leaf E were lower (5.1 and 5.5 mmol H_2O m^{-2} s^{-1}, respectively) and were reached in the morning (11:30 h), whereas leaf E declined more sharply under White control net (Fig. 3-7A). Daily mean leaf E was highest under Blue net (5.2 mmol H_2O m^{-2} s^{-1}) and lowest under White control and Red nets (4.6 and 4.5 mmol H_2O m^{-2} s^{-1}, respectively, $p<0.05$) (Table 3-1). For all net treatments, maximum values of leaf C_i (290 – 295 μmol CO_2 mol^{-1}) were observed in the early morning and afternoon (Fig. 3-7B). From early morning (8:30 h) leaf C_i decreased sharply under Red, Grey and White control nets, reaching minimum values at midday (12:50) under White control net (271 μmol CO_2 mol^{-1}). Under Blue net, leaf C_i decreased more gradually and the minimum value was 280 μmol CO_2 mol^{-1} (Fig. 3-7B). Daily mean leaf C_i was highest ($p<0.001$) under Blue net (285 μmol CO_2 mol^{-1}) compared to White control (279 μmol CO_2 mol^{-1}) and Red (282 μmol CO_2 mol^{-1}) nets (Table 3-1). No differences on daily mean WUE were observed ($p>0.05$) among net treatments (Table 3-1). Nevertheless, daily mean leaf IWUE under Grey and Blue was significantly ($p<0.01$) lower than compared to White control net (39 vs. 43.7 μmol CO_2 mol^{-1} H_2O, respectively) (Table 3-1), whereas daily mean leaf IWUE under the Red net did not differ from the Grey one, but was greater than Blue net (Table 3-1). A linear, negative relationship was found between leaf C_i and IWUE, where differences of C_i among net treatments are explained about 92% by variations in IWUE (Fig. 3-8).

Leaf gas exchange and chlorophyll fluorescence under controlled light conditions

Table 3-4 shows the gas exchange and chlorophyll fluorescence parameters for two periods in the day: mid-morning (9:30 – 11:00 h) and midday (11.45 – 13:30 h) and measured at controlled PPFD (1050 and 1450 µmol m^{-2} s^{-1}, respectively). Leaf A_n under Blue, Grey and Red shade nets was 2 - 3 µmol CO_2 m^{-2} s^{-1} higher than leaves grown under White net ($p<0.01$; Table 3-4) at mid-morning. Nevertheless, at midday leaf A_n in Blue and Grey leaves were higher ($p<0.001$) than White and Red nets by up to 2 µmol CO_2 m^{-2} s^{-1} of difference (Table 3-4). Leaf g_s was lowest ($p<0.001$) for White net (0.14 - 0.10 mol m^{-2} s^{-1}) and highest for Blue net (0.19 - 0.17 mol m^{-2} s^{-1}) at mid-morning and midday, respectively. At mid-morning, leaf g_s did not differ between Red and Grey nets, however at midday leaf g_s under Grey net was up to 10% higher than Red net ($p<0.001$). Leaf g_s under Blue net was 10 and 25% greater than those under Red net for mid morning and midday, respectively (Table 3-4). Although all net treatments received a similar amount of PPFD, $VPD_{leaf\text{-}air}$ differed significantly among them. At mid-morning, $VPD_{leaf\text{-}air}$ under Grey net was lower than White control net, but there were no differences among Red, Blue and White control nets. At midday, $VPD_{leaf\text{-}air}$ was significantly lowest under Blue net, but there were no differences among Red, Grey and control White nets (Table 3-4).

Efficiency of light harvesting by PSII, as measured by F_v'/F_m' was higher under Blue and Grey nets compared to control White net, in both moments of the day (mid-morning and midday). At midday, Blue net presented greater F_v'/F_m' values than Red net. Differences in electron transport rate (ETR) and quantum efficiency of PSII (Φ_{PSII}) among net treatments were also found. They were highest under Blue net (Table 3-2), but the differences were only significant at mid-morning. At the same time, leaf *q*P was slightly affected by net treatments. Under Blue net, *q*P was higher than Grey net, but did not differ from Red and White nets (Table 3-2). Leaf A_n and g_s

were closely and positively related to changes in F_v'/F_m'. At midday, the relationship was higher than mid-morning (Figs 3-9 A and B).

Leaf characteristics, leaf water potential and soil water status

Leaves grown under Blue and Grey nets presented greater chlorophyll content (54.4 SPAD units) than Red net (51.5 SPAD units), but not than the control (White net) (Table 3-5). LMA values under Grey and Blue nets were lower than control White net (11.2 vs. 12.3 mg cm^{-2}, respectively; $p<0.001$). However, there were no differences in LMA among Red and control White net, while LMA under the Red was statistically lower than the Blue net (Table 3-5). Leaf area under Blue net was highest (22.7 cm^2; $p<0.001$) compared to control and other colored shade nets (19.2 cm^2 on average). Daily leaf water potential (Ψ_{leaf}) did not differ among net treatments (Fig. 3-10 A). The minimum value of Ψ_{leaf} was reached at midday (12:00 h) and tended to be slightly lower under Blue net (-1.7 MPa) compared to control White net (-1.5 MPa). Soil water evaporation rate estimated from pots drying did not differ significantly until 8:00 h (Fig. 3-10B). However, at midday, soil water evaporation from pots under White net was 9, 16, 19% higher than Red, Blue and Grey nets, respectively. In the warmest part of the day (12:00 – 16:00 h). Grey net was conserving more soil water compared to all colored shade nets, especially at 16:00 h. where soil water evaporation rate under Grey net was 7 mg H_2O cm^{-2} h^{-1} (20%) lower than control and other colored shade nets (Fig. 3-10B).

DISCUSSION

Stomatal conductance regulation

Significant reduction of midday PPFD by Blue and Grey shade net compared to White net (control), can lead to a reduction in $VPD_{leaf\text{-}air}$ and an increment in leaf g_s (Figs. 3-2 and 3-3A). Reduction of $VPD_{leaf-air}$ by shade netting can be a consequence of reduced leaf and air temperature and increment in relative humidity (Iglesias & Alegre, 2006). In our study, there were no differences in air temperature and relative humidity among net treatments (data not shown), but leaves under Blue and Grey nets were on average 0.5 °C cooler than under the White control net (Table 3-1). These results agree with findings reported for apple (Smit *et al.,* 2008) and citrus trees (Jifon & Syvertsen, 2003), which demonstrated that reduction of midday PPFD by moderate neutral black shading significantly incremented leaf g_s as a consequence, primarily, of a reduction in T_{leaf} associated to a reduction in $VPD_{leaf-air}$. We found a linear and negative relationship between g_s and $VPD_{leaf-air}$ (Fig. 3-5). After mid-morning (10:30 – 11:30 h) and midday (12:50 – 14:00 h) changes in g_s among net treatments were explained by variation in $VPD_{leaf-air}$ by 62% and 72% respectively (Fig. 3-5C), confirming the typical depletion observed in apple leaf g_s by increasing $VPD_{leaf-air}$ and which is more marked as VPD increased (Warrit *et al.,* 1980; Flore *et al.,* 1985). In the early morning (between 8:30 and 9:30) when $VPD_{leaf-air}$ ranged 1.5 – 2.0 kPa, leaf g_s was not significantly correlated to changes in $VPD_{leaf-air}$ in any net treatment (Fig. 3-5 A; Table 3-2), while after midmorning (when $VPD_{leaf-air}$ exceeded ~ 2.0 kPa), leaf g_s decreased linearly as $VPD_{leaf-air}$ increased for all colored shade nets (Fig. 3-5B). These results are in accordance with previous studies carried out in 'Gold spur', 'Empire', 'Fuji' and 'Braeburn', which showed that leaf g_s was not closely linked to VPD variations under cooler ambient conditions or with lower values of $VPD_{leaf-air}$. On the other hand, it decreased linearly under warmer conditions and/or high values of VPD, and after $VPD_{leaf-air}$ exceeded a certain threshold which was estimated between 1.5 – 2.0 kPa (Cheng & Luo, 1997; Francesconi *et al.,* 1997; Dragoni *et al.,* 2004; Massonet *et al.,* 2007). At midday, regression coefficients between g_s and $VPD_{leaf-air}$ were significantly lower under Blue and Grey nets, indicating that, under these nets, environment conditions were less limiting leaf g_s by $VPD_{leaf-air}$. Therefore, Blue and Grey shade nets seem more effective in controlling leaf g_s depletion in apple orchards, and appear most beneficial under climate conditions leading to extreme VPD values. On the contrary, under Red shade net leaf g_s was significantly lower than Blue and Grey nets (Fig 3-4A) and more readily limited by changes in $VPD_{leaf-air}$ (Table 3-2). Differences in leaf g_s between Red and Grey can be attributed to a marked reduction of T_{leaf} and $VPD_{leaf-air}$ associated to PPFD reduction (Fig 3-2). However, leaf g_s differences between Red and Blue nets can not be explained by changes in PPFD, because during the whole day Red and Blue reduced at the same proportion the amount of PPFD compared to the White net (Fig.3-3A and Table 3-1). Differences in spectral light transmission among colored shade nets could be involved in these responses (Fig. 3-2). Light spectra analysis carried out under field conditions demonstrated that, under the Blue net, radiation was richer in blue photons compared to the Red net (Fig. 3-2). Blue light has always been found more effective than red light in the stimulation of stomata opening as well as in preventing stomatal closure (Farquhar & Sharkey, 1982). Therefore, Blue net would be improving leaf g_s compared to Red net by direct effect of blue light on stimulation of stomatal aperture as has widely been proposed in the literature (Shimazaki *et al.,* 2007; Taiz & Zeiger, 2009). A further explanation is relative to the NIR component of sunlight. NIR transmission was also significantly altered by colored shade nets. The Grey and Blue nets significantly reduced the amount of NIR radiation in comparison to Red and White (control) nets (Fig. 3-2). Although leaves in fruit trees present reduced absorbance of NIR radiation (~5%) (Baldini *et al.,* 1997),excessive NIR transmission can also be contributing to heat load which commonly leads to increases in leaf temperature, especially in protected crops grown under high radiation (Hemming *et al.,* 2006). Since under Red net daily patterns of T_{leaf} and $VPD_{leaf-air}$ were significantly greater than Blue and especially Grey net (Fig 3-3B), the differences in NIR transmission among colored shade nets might be another factor explaining why we found reduced leaf g_s under Red shade net (Fig. 3-4 A).

On the other hand, when analyzed under controlled PPFD (i.e. similar light quantity and quality) during the two periods of the day (midmorning and midday), leaf g_s was highest under Blue net and lowest under the control White net (Table 3-4), indicating the participation of complementary factor (s) on leaf g_s regulation under colored shade nets. Although $VPD_{leaf\text{-}air}$ differed among colored shade nets, it was not clearly linked to g_s as under ambient sunlight conditions (Table 3-4). For example, mid-morning leaf g_s under Blue net was higher than Red and White control nets, however $VPD_{leaf\text{-}air}$ did not differ among those treatments (Table 3-4). It has been widely demonstrated that leaf g_s in apple trees is also influenced by plant and soil water status (Lakso, 2003). In our study, trees were planted in pots. Calderón-Zavala *et al.,* (2004) suggested that potted apple trees could suffer short-term water stress when exposed to extreme temperature conditions, although well irrigated during the day. Leaf water potentials (Ψ_{leaf}) did not differ among net treatments during the day (Fig. 3-10A). Midday values of Ψ_{leaf} ranged between -1.53 to -1.68 MPa, similar to values estimated for well irrigated apple trees under field and pot conditions (Lakso, 2003; Atkinson *et al.,* 2000), therefore in our experiment plant water status should not have caused leaf g_s changes under the various colored shade nets. Indeed, the minimum midday Ψ_{leaf} value was reached under the Blue net (-1.68 Mpa), which presented the maximum values of leaf g_s and transpiration rates at that time (Fig. 3-7A). Hydraulic resistance is high in apple trees, therefore diurnal leaf E increases normally are associated with greater diurnal decrease in leaf water potential (Lakso, 2003).

Daily leaf E in apple trees is strongly influenced by atmospheric conditions (VPD and PPFD), thus high leaf E is normally linked to high VPD, however beyond a certain threshold the relation between leaf E and VPD becomes non linear due to feedback control of leaf E by leaf g_s (Cheng & Luo, 1997; Giuliani *et al.,* 1997). In this study, reduction of PPFD and $VPD_{leaf\text{-}air}$ by Grey and Blue nets led to significant increments in leaf E (Fig. 3-7), because higher leaf g_s is associated to leaf E increment. Reduction of light levels by particle films incremented leaf g_s and E but reduced the WUE in apple trees (Glenn *et al.,* 2003), whereas shading reduced leaf E, g_s and incremented the WUE in 'Gala' apple trees (Gindaba & Wand, 2007). In the present study we did not find differences in WUE among colored shade nets (Table 3-1), probably because higher leaf g_s and E were also coupled with greater leaf CO_2 assimilation (Fig. 3-3 A and B).

Although microclimate conditions under Grey and Blue nets (reduction of PPFD, $VPD_{leaf\text{-}air}$ and probably NIR) increase plant transpiration, we observed an opposite effect on soil water evaporation rate estimated in soil samples from pots (Fig. 3-10 B). At midday, soil water evaporation rate was more effectively reduced under Blue and Grey nets, whereas later in the afternoon reduction of soil water evaporation from pots was more significant under Grey net (Fig. 3-12 B). Improving soil moisture by shading has been suggested in apple orchards but without results up to date (Smit *et al.,* 2008). Although no differential effect of colored shade nets on soil water status was reflected on changes in Ψ_{leaf}, the soil water conserving effect observed under Blue and Grey nets could be another factor involved in improving leaf g_s under these nets, probably by a feedback mechanism. This might contribute to the reduction in leaf g_s depletion observed in apple trees under high temperature and radiation.

Stomatal conductance and photosynthetic capacity

Diurnal patterns of leaf g_s and photosynthesis rate (A_n) were similar for all colored shade nets, peaking at mid-morning (9:30 h) and then declining during the rest of the day (Fig. 3-4 A and B). These patterns are similar to those observed in 'Gold Spur' apple trees under field conditions, where maximum g_s and A_n were reached around 9:00 hour (Cheng & Luo, 1997). Leaf g_s was strongly correlated to A_n during the three moments of the day reported: here early morning, mid-morning and midday (Fig. 3-6; Table 3-3), confirming the feedback regulation of photosynthesis by leaf g_s as previously suggested in apple trees under field conditions (Lakso, 2003; Wünsche *et al.,* 2005). Furthermore, it was confirmed that, under climate conditions with lower $VPD_{leaf\text{-}air}$ (early morning in our case) where apple leaf g_s is less influenced by $VPD_{leaf\text{-}air}$ (Fig. 3-5), leaf g_s is more related to changes in photosynthesis rate (Francesconi *et al.,* 1997). However, we did not detect differences in leaf A_n among colored shade nets (Fig. 3-4 B; Table 3-1) and regression coefficients between A_n and g_s differed among colored shade nets (Table 3-3). These results indicate that a non-stomatal limitation mechanism would also be participating in photosynthesis regulation under colored shade nets. Leaf structural characteristics, mainly palisade thickness, play an important role on photosynthetic capacity, thus leaves with greater palisade thickness present higher Rubisco activity and CO_2 fixation (Oguchi *et al.,* 2003). In previous experiments we demonstrated that leaves under Red, Blue and Grey nets presented lower palisade cell development compared to those from control (White net) (see Chapter II in this Dissertation). Leaves with reduced palisade cell development frequently show lower leaf mass per area (LMA): it is likely that Red, Blue and Grey leaves were more limited in their photosynthetic machinery than the leaves grown under White net (control). Indeed, in this study leaves under Blue and Grey nets presented lower LMA compared to control (Table 3-5). Therefore, although Blue and

Grey nets increased leaf g_s compared to control, photosynthetic capacity was statistically similar among net treatments.

Massonet *et al*. (2007) found a relation between stomatal limitation and intrinsic water use efficiency (IWUE, i.e. the ratio between A_n/g_s); in apple leaves with high IWUE photosynthesis limitation by g_s prevails, whereas in those with lower IWUE, non stomatal limitation factors (palisade thickness, Rubisco activity, etc) prevail. The results of the present study are in accord with this interpretation: the IWUE values under Blue and Grey nets were significantly lower, thus indicating that An was less limited by g_s under Blue and Grey nets compared to control and Red net (Table 3-1). If g_s limits An, decrease of intercellular CO_2 concentration (C_i) must occur (Farquhar & Sharkey, 1982). Daily mean leaf C_i was highest under Blue compared to White and Red nets (Fig. 3-7B; Table 3-1), indicating a possible stomatal limitation of CO_2 diffusion under the latter nets. Interestingly, under Blue net C_i decrease was less sharp during the day and was more constant compared to other colored shade nets (Fig. 3-7). These results can be explained by the effect of blue light on increasing stomata opening which increases CO_2 diffusion to intercellular spaces, therefore reducing stomatal limitations to photosynthesis (Loreto *et al.,* 2009). Furthermore, we found a close negative relationship between IWUE and C_i (Fig. 3-8), thus leaves that presented higher IWUE such as those grown under White and Red nets presented lower C_i concentration due to parallel reduction of leaf g_s.

Another important factor on photosynthetic capacity is the light availability. In 'Royal Gala' apples Gindaba & Wand (2007) found significant decreases of leaf A_n and g_s by reduction of 35% PPFD with black shade nets. They concluded that reduction of PPFD by shading induces morpho-functional changes in leaves and can lead to decreased photosynthetic potential. As discussed above, in our study reduction of PPFD induced morphological changes that limited leaf photosynthetic capacity; even so, leaf A_n tended to be higher under Blue and Grey nets, even though they reduced PPFD by 25 – 35% respect to the control (Fig 3-3 A). Furthermore, under Blue net leaf A_n was always greater than Red net and in both cases they received the same quantity of PPFD (Fig. 3-3A).Under all colored shade nets the maximum PPFD value was between 900 – 1100 µmol m^{-2} s^{-1}, i.e., nearly saturating. Therefore, it is not surprising that leaf A_n under colored shade nets was similar or higher than control (White net), whose maximum PPFD was 1500 µmol m^{-2} s^{-1}. In this context, colored shade nets were more efficient in light use for CO_2 assimilation, which was confirmed when leaf A_n under similar PPFD levels was evaluated (Table 3-4). Leaf A_n under Blue, Grey and Red shade nets was 2 - 3 µmol CO_2 m^{-2} s^{-1} higher than for the control in mid-morning. Furthermore, midday A_n values in Blue and Grey leaves were also significantly higher than in Red ones. Although leaf A_n differences were related to leaf g_s changes, fluorescence parameters were also significantly affected. During both mid-morning and midday, leaf F_v'/F_m' was significantly greater under Blue and Grey nets compared to control (Table 3-4). Leaf F_v'/F_m' represents the efficiency of light harvesting by open reactions centers of PSII (Genty *et al.,* 1989). Leaves under Blue and Grey shade nets presented more chlorophyll content than control and Red net (Table 3-5), which would explain the higher light harvesting efficiency observed under Blue and Grey nets. Interestingly, at mid-morning electron transport rate (ETR) and partial quantum yield (Φ_{PSII}) were highest under Blue net (Table 3-5). Φ_{PSII} measures the proportion of light absorbed by chlorophyll associated with PSII that is used in photochemistry, while ETR is and indicator of overall photosynthetic capacity (Maxwell & Johnson, 2000). Therefore these results demonstrate a photochemical mechanism (non stomatal) increasing leaf photosynthetic capacity under Blue net. Regression analysis demonstrated that ETR and Φ_{PSII} were closely linked to A_n and g_s among colored nets (data not shown). Indeed changes in leaf An and g_s were explained from 60 to 70% by changes in Fv'/Fm' (Fig. 3-9). These results could be indicating a feedback regulation of leaf g_s by photochemical processes under colored shade netting which up to date aye not yet reported.

CONCLUSIONS

Under high temperature and radiation conditions, colored shade nets increases leaf stomatal conductance in apple trees, but the effects was differential depending on net color. Blue and Grey nets are more effective than Red net in mitigating daily leaf stomatal depletion in apple trees. Covering apple orchards with Grey and Blue nets could be a useful cultural practice in apple production environments where conditions of excessive radiation and high temperatures might be limiting stomatal conductance in apple trees, with ensuing effects on tree/orchard yield potential.

Increases of apple leaf g_s by Blue and Grey shade nets would be linked to reduction of high sunlight stress resulting in mitigation of high T_{leaf} and $VPD_{leaf\text{-}air}$, but also due to changes in spectra light quality conditions (more blue light vs. less red and NIR light transmission). Soil water status and leaf photochemical process (ETR, Fv'/Fm') would be also involved by probably feed-back mechanisms.

Water use efficiency was not affected significantly by colored shade nets. However, light conditions (greater reduction of PPFD and NIR) under Grey net would be helpful in conserving soil moisture, therefore providing an interesting potential to reduce the irrigation needs in orchards especially under drought conditions.

Leaf photosynthesis under Blue nets seems to be less limited by stomatal conductance, probably by a direct effect of blue light on stimulation of stomatal opening. Net CO_2 assimilation was not reduced by color shade nets, indeed it appeared incremented under Blue and Grey shade nets which were more efficient in light use for CO_2 assimilation. These responses can be explained by stomatal (higher stomatal conductance) and non-stomatal (higher photochemical efficiency) regulatory mechanisms.

TABLES AND FIGURES

Table 3-1. The effect of colored shade nets on daily mean (8:30 a.m. – 16:15 p.m.) value of gas exchange parameters in 'Fuji' apple leaves. Measurements were made under ambient conditions on 8 and 9 July, 2010.

Parameters	Net treatments				*p-value*
	White (control)	Red	Blue	Grey	
PPFD (μmol m^{-2} s^{-1})	1241.5a[1]	960.8b	941.4b	838.0c	***
T_{air} (°C)	34.9	34.8	34.8	34.9	NS
$T_{leaf\text{-}air}$ (°C)	0.08a	-0.2ab	-0.3b	-0.4c	***
$VPD_{leaf\text{-}air}$ (kPa)	3.3a	3.2ab	3.1b	3.0b	**
g_s (mol m^{-2} s^{-1})	0.15b	0.15b	0.19a	0.17ab	*
A_n (μmol CO_2 m^{-2} s^{-1})	6.7	6.5	7.4	7.1	NS
C_i (μmol CO_2 mol^{-1})	279.0b	282.3b	285.7a	285.2ab	***
E (mmol H_2O m^{-2} s^{-1})	4.6b	4.5b	5.2a	4.9ab	*
WUE (μmol CO_2 $mmol^{-1}$ H_2O)	1.49	1.48	1.45	1.46	NS
IWUE (μmol CO_2 mol^{-1} H_2O)	43.7a	42.2ab	39.3c	39.9bc	**

[1] Mean separation within rows by the Student-Newman-Keuls test; n= 12. ^{NS}; *; **; *** non significance and significance at $p<0.05$, 0.01 and 0.001, respectively.

Table 3-2. Regression coefficients between leaf stomatal conductance and leaf-to-air vapour pressure deficit at three moments during the day: early morning (8:30 h), midmorning (10:30 h) and midday (12:50 h).

Periods	Net treatments			
	White (control)	Red	Blue	Grey
Early morning	0.14^{NS}	0.05^{NS}	0.24^{NS}	0.03^{NS}
Midmorning	0.66**	0.65**	0.62**	0.41*
Midday	0.89***	0.86***	0.71***	0.57**

^{NS}; *; **; *** non significance and significance at $p<0.05$, 0.01 and 0.001, respectively.

Table 3-3. Regression coefficients between photosynthetic rate and leaf stomatal conductance at three moments during the day: early morning (8:30 h), midmorning (10:30 h) and midday (12:50 h).

Periods	Net treatments			
	White (control)	Red	Blue	Grey
Early morning	0.90***	0.72***	0.53**	0.71***
Midmorning	0.74***	0.83***	0.67**	0.88***
Midday	0.44*	0.91***	0.81***	0.77***

^{NS}; *; **; *** non significance and significance at $p<0.05$, 0.01 and 0.001, respectively (n=12).

Table 3-4. The effect of colored nets on gas exchange and chlorophyll fluorescence parameters measured in 'Fuji' apple leaves during two periods in the day. Leaf measurements were repeated during four summer days (23, 25, 28 and 29 June, 2010) and averaged.

Parameters	Net treatments				*p-value*
	White (control)	Red	Blue	Grey	
Mid-morning					
A_n (µmol CO_2 m^{-2} s^{-1})	10.7b[1]	12.4a	13.9a	12.7a	**
C_i (µmol CO_2 mol^{-1})	237.7	241.3	247.0	241.9	NS
g_s (mmol m^{-2} s^{-1})	0.14c	0.17b	0.19a	0.17b	***
VPD $_{\text{leaf-air}}$ (kPa)	2.2a	2.1ab	2.1ab	2.0 b	**
F_v'/F_m'(dimensionless)	0.50b	0.52ab	0.53a	0.53a	*
Φ_{PSII} (dimensionless)	0.31b	0.31b	0.34 a	0.31b	*
ETR (µmol m^{-2} s^{-1})	140.4b	149.3b	159.1a	141.6b	*
qP(dimensionless)	0.62ab	0.60ab	0.63a	0.59b	*
Midday					
A_n (µmol CO_2 m^{-2} s^{-1})	7.1b	7.9b	10.4a	9.2a	***
C_i (µmol CO_2 mol^{-1})	238.0	236.7	237.8	252.1	NS
g_s (mol m^{-2} s^{-1})	0.10d	0.11c	0.14a	0.12b	***
VPD $_{\text{leaf-air}}$ (kPa)	3.3a	3.2a	3.1b	3.2a	***
F_v'/F_m'(dimensionless)	0.39c	0.41b	0.44a	0.42ab	***
Φ_{PSII} (dimensionless)	0.21	0.22	0.24	0.23	NS
ETR (µmol m^{-2} s^{-1})	140.4	142.1	153.4	149.0	NS
qP (dimensionless)	0.54	0.52	0.54	0.53	NS

[1] Mean separation within rows by the Student-Newman-Keuls test; n= 6. ^{NS}; *; **; *** non significance and significance at $p<0.05$, 0.01 and 0.001, respectively.

Table 3-5. The effect of colored nets on leaf chlorophyll content, single leaf area and leaf dry mass area per unit (LMA) in ‘Fuji’ apple trees.

Net treatments	Chlorophyll content (SPAD units)	Leaf area (cm^2)	LMA (mg cm^{-2})
White (control)	52.7ab[1]	18.6b	12.3a
Red	51.5b	19.7b	11.8ab
Blue	54.4a	22.7a	11.1c
Grey	54.6a	19.5b	11.4bc
p-value	*	***	***

[1] Mean separation within rows by the Student-Newman-Keuls test; n= 20 for leaf chlorophyll content or 40 for leaf area and leaf mass area ratio measurements. ^{NS}; *; **; *** non significance and significance at $p<0.05$, 0.01 and 0.001, respectively.

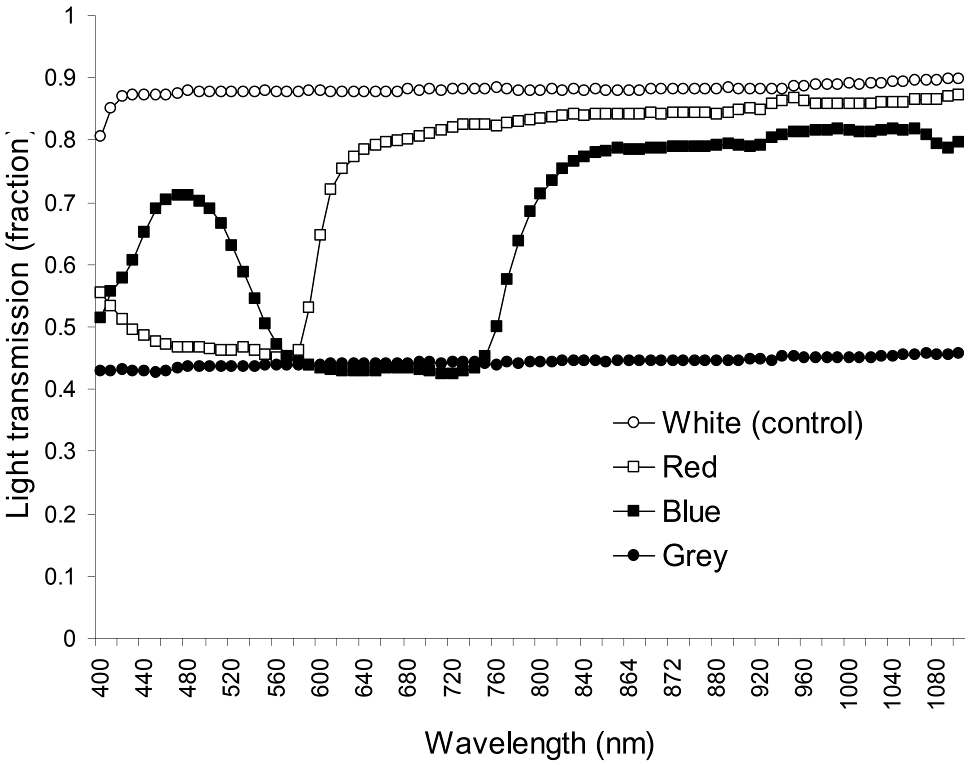

Fig. 3-1. Spectral light transmission of Grey, Blue and Red shade nets respect to White (control) net.

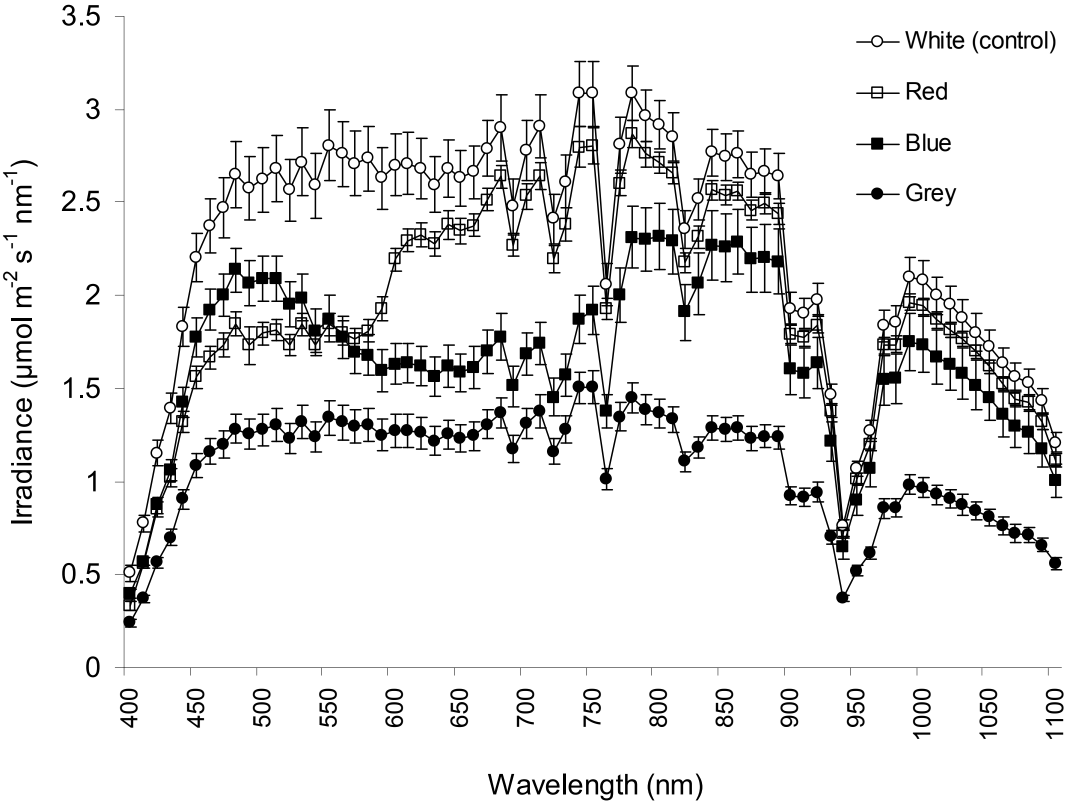

Fig. 3-2. The effect of colored shade nets on mean daily spectral irradiance measured at three times of the day (3 h before solar noon, solar noon and 3 h after solar noon). Each point represents the mean ± SE of four reading.

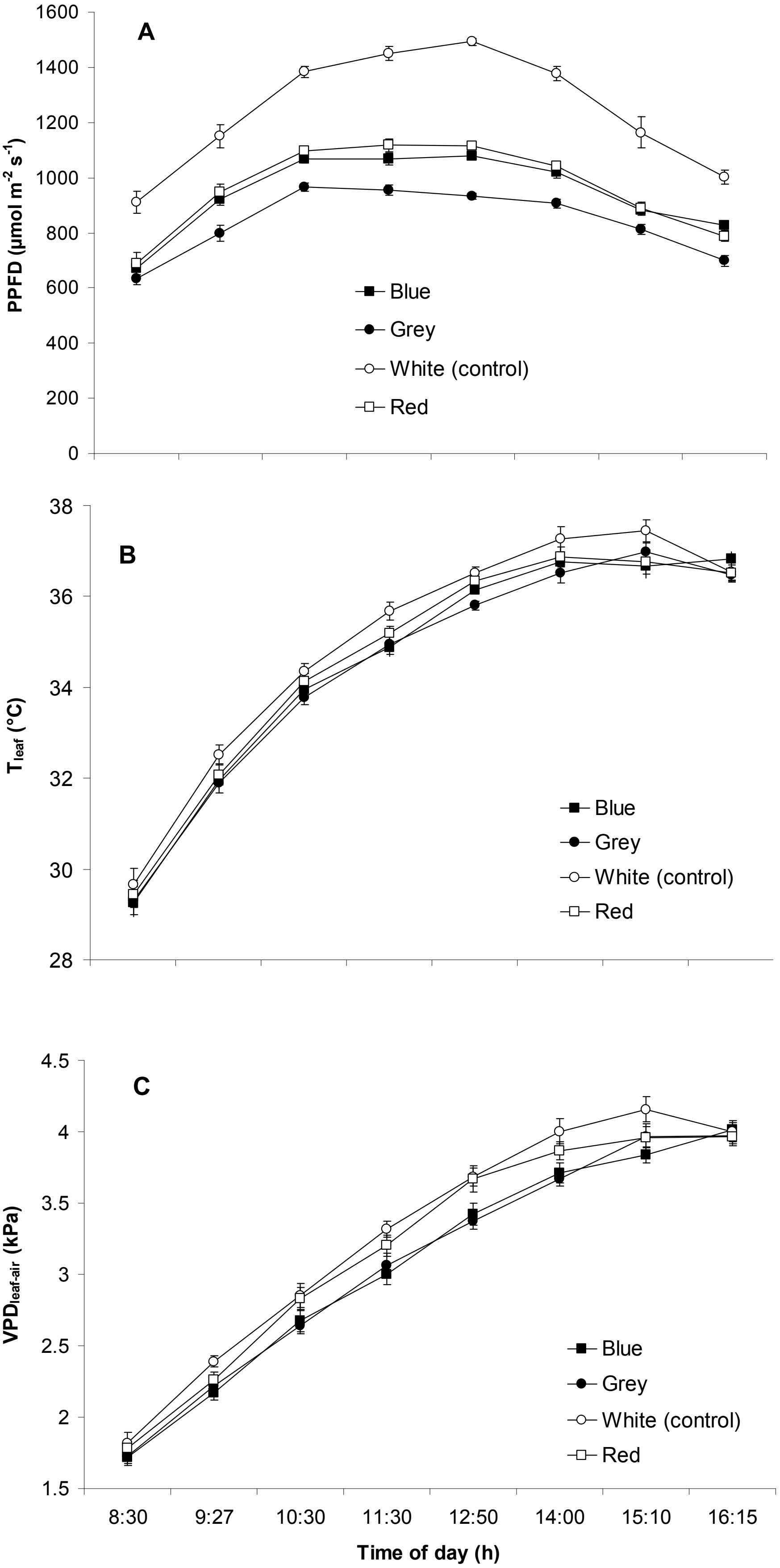

Fig. 3-3. The effect of colored shade nets on daily course of (A) photosynthetic photon flux density, PPFD, (B) leaf temperature, T_{leaf} and (C) leaf-to-air vapour pressure deficit, $VPD_{leaf\text{-}air}$, in 'Fuji' apple trees. Each point represents the mean ± SE of 12 leaves measurements on July 8 and 9, 2010.

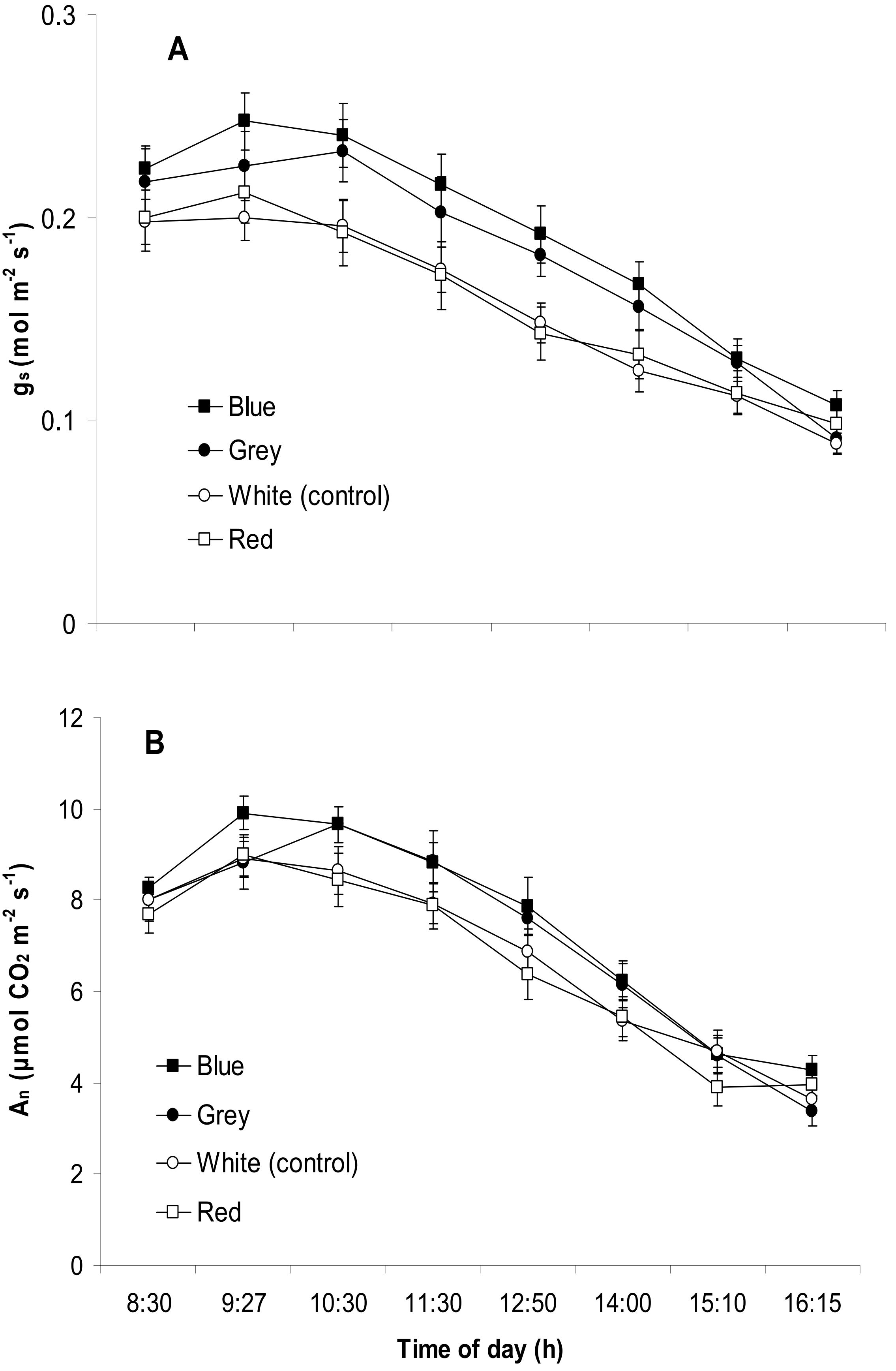

Fig. 3-4. The effect of colored shade nets on daily course of (A) leaf stomatal conductance, g_s, and (B) leaf photosynthesis rate, An, in ‘Fuji’ apple trees. Each point represents the mean ± SE of 12 leaves measurements on July 8 and 9, 2010.

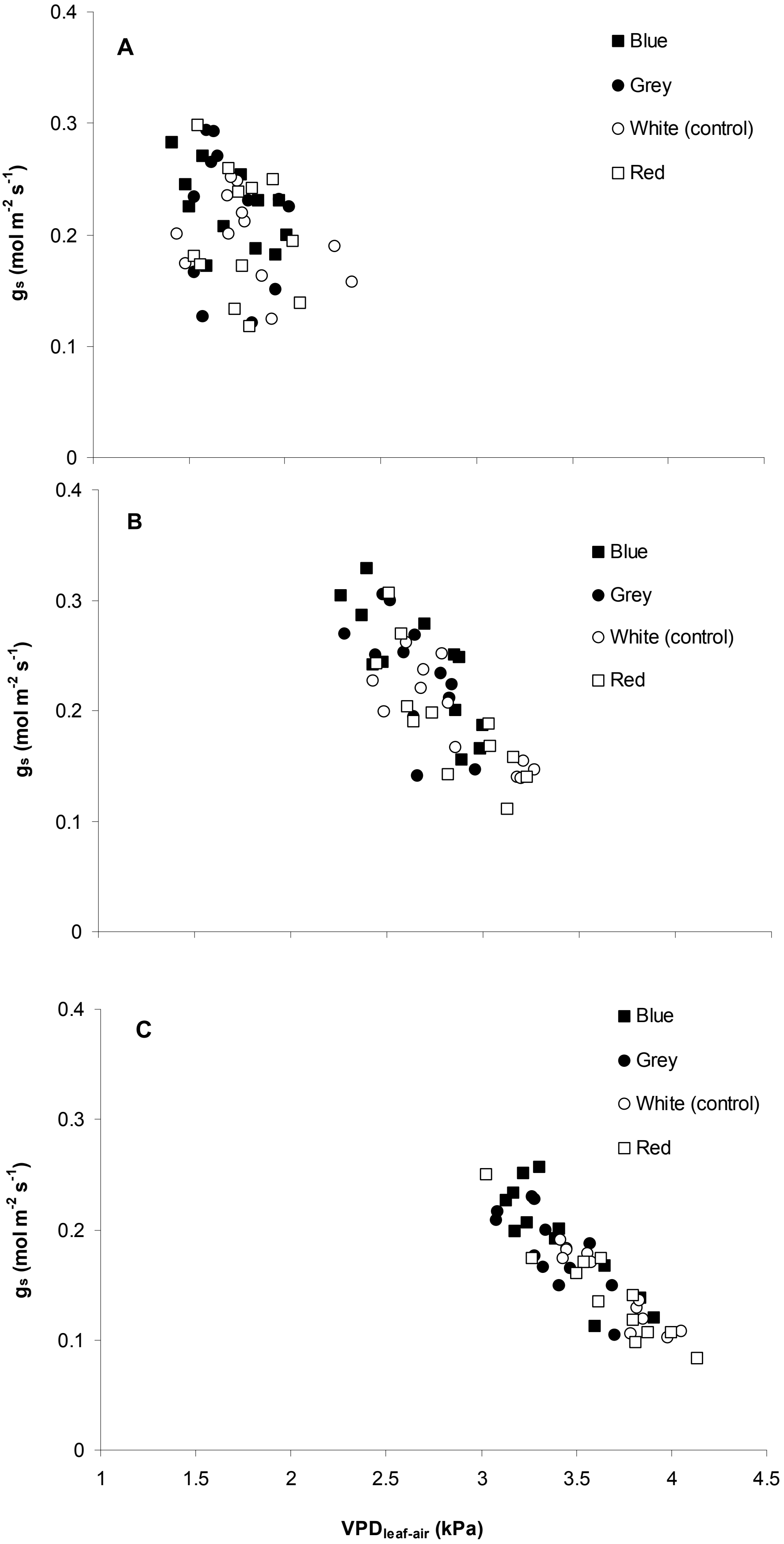

Fig. 3-5. Relationship between leaf stomatal conductance (g_s) and leaf-to-air vapour pressure deficit ($VPD_{leaf-air}$) at early morning (A), after midmorning (B) and midday (C) in 'Fuji' apple leaves grown under different colored shade nets. Linear equation: $gs = a + b\ VPD_{leaf}$ (n=48)

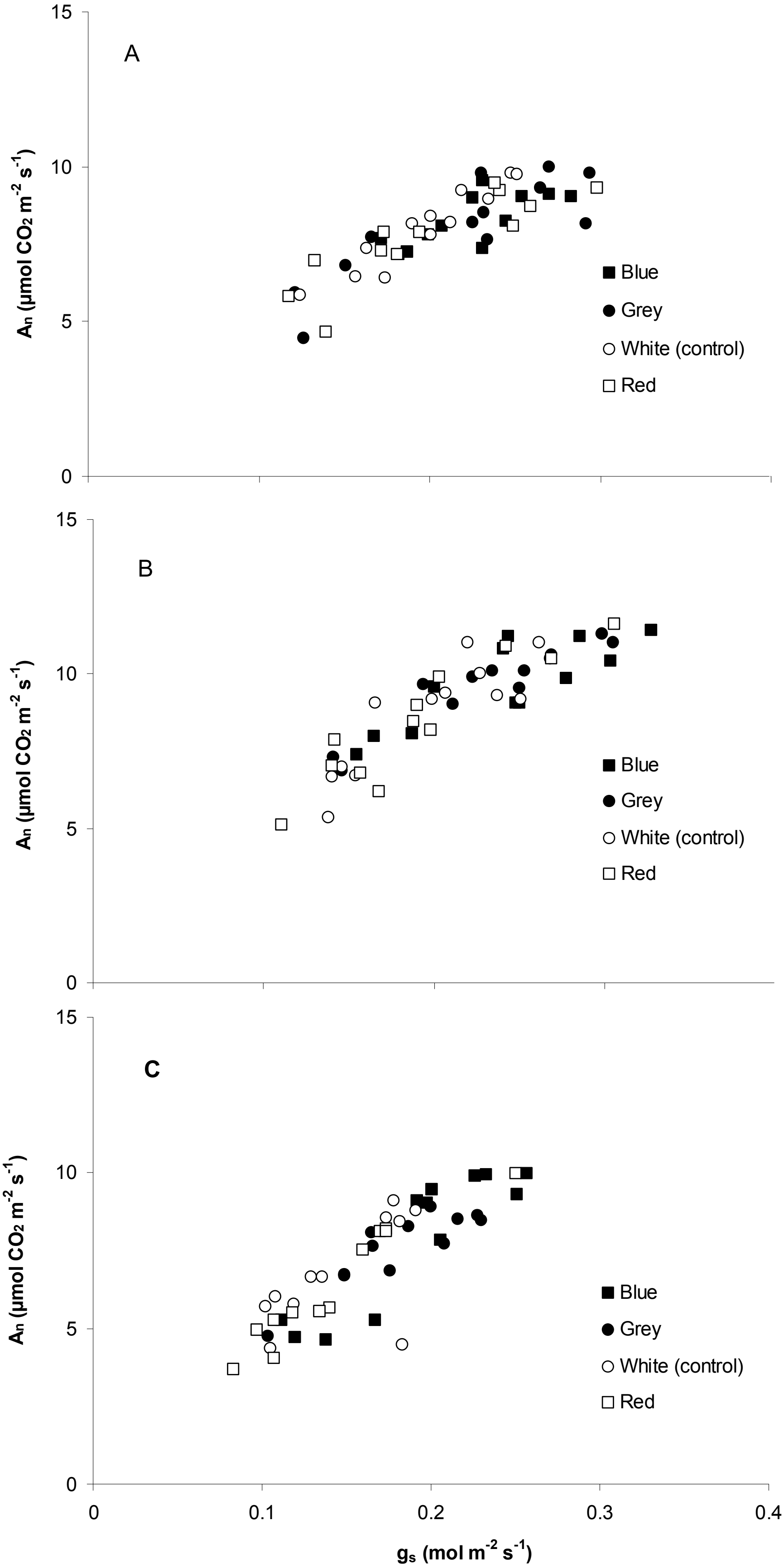

Fig. 3-6. Relationship between leaf photosynthesis rate (A_n) and leaf stomatal conductance (g_s) at early morning (A), midmorning (B) and midday (C) in 'Fuji' apple leaves grown under different colored shade nets. Lineal equation: $A_n = a + b\ g_s$ (n=48)

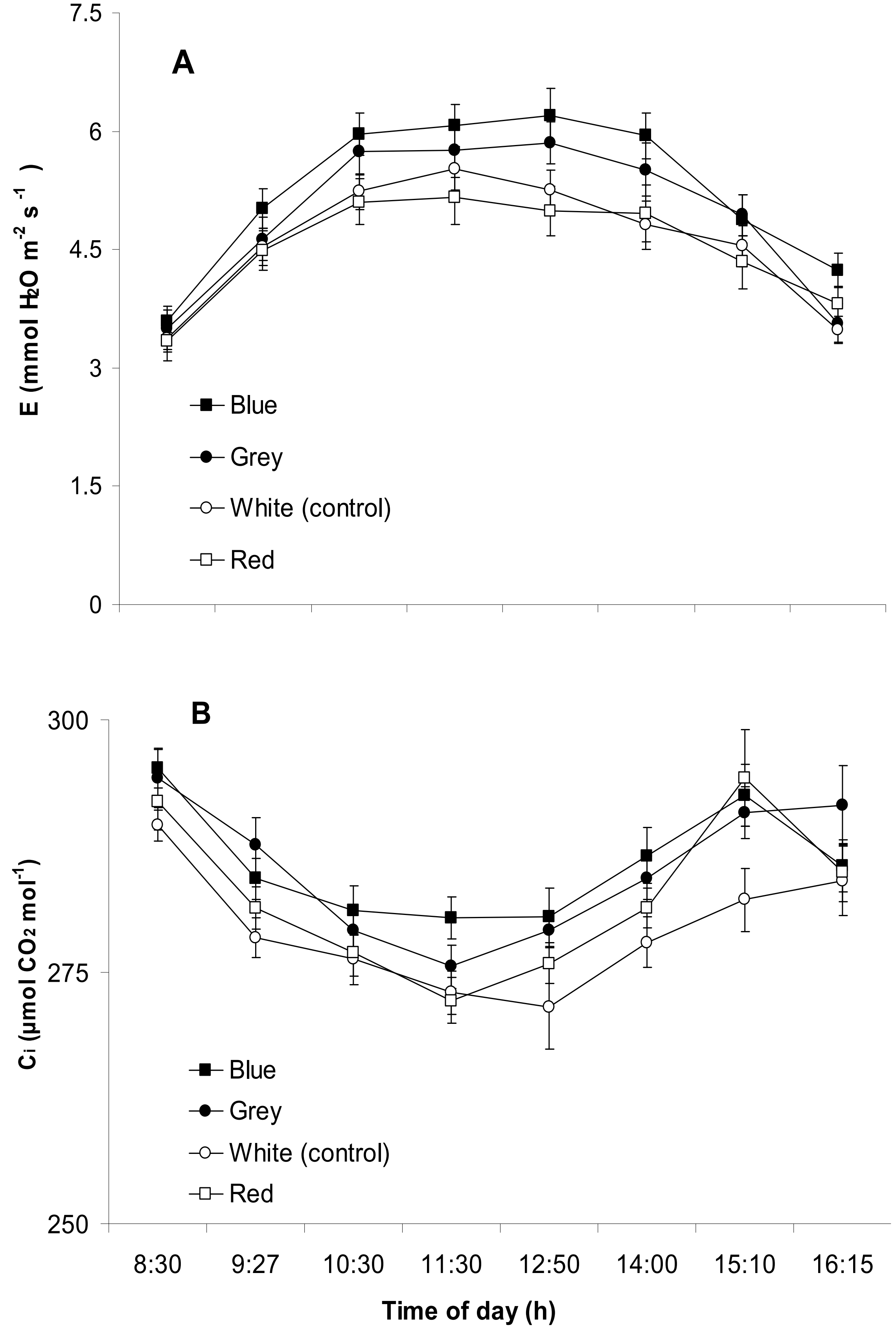

Fig. 3-7. The effect of colored shade nets on daily course of (A) transpiration rate, E, and (B) intercellular carbon dioxide concentration, C_i, in 'Fuji' apple leaves. Each value represents the mean ± SE of 12 leaves measurements on July 8 and 9, 2010.

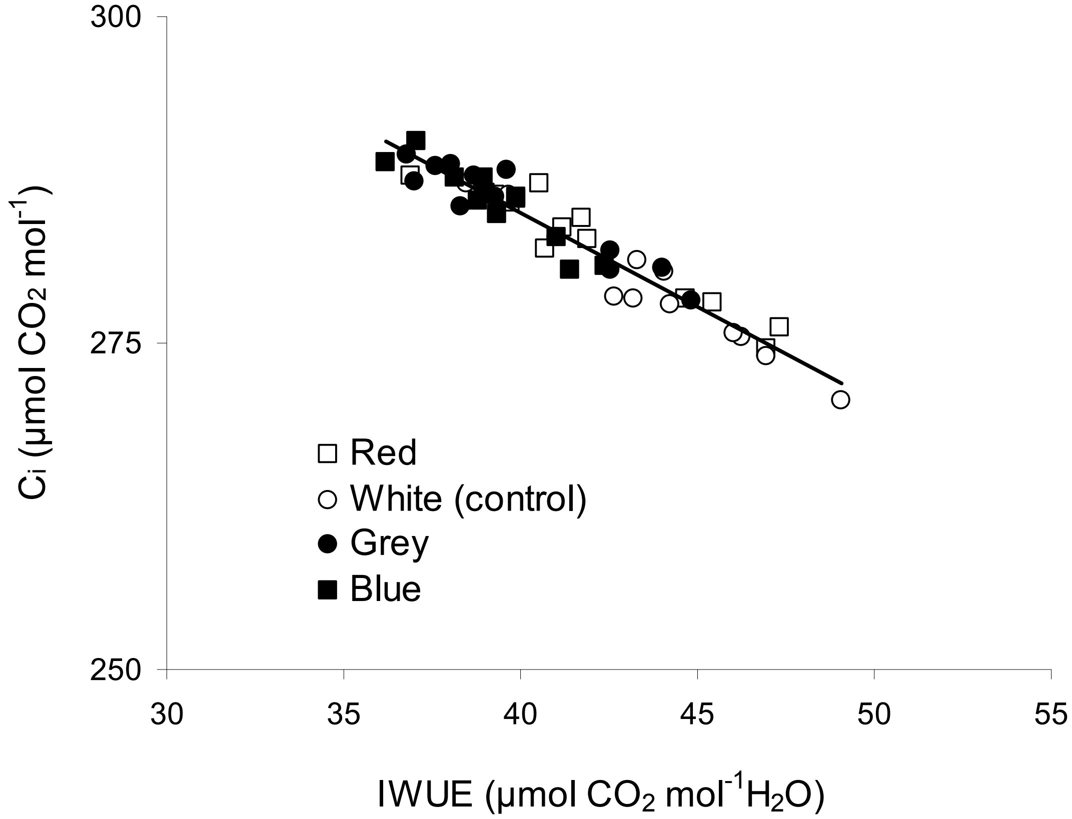

Fig. 3-8. Relationship between intercellular concentration of carbon dioxide (C_i) and intrinsic water use efficiency (IWUE) and in 'Fuji' apple leaves grown under different colored shade nets. Each point represents the daily mean (8:30 – 16:15) measurements on July 8 and 9, 2010. Linear regression equation is [C_i = 342.4 - 1.4 IWUE] (n=48, r^2=0.92***).

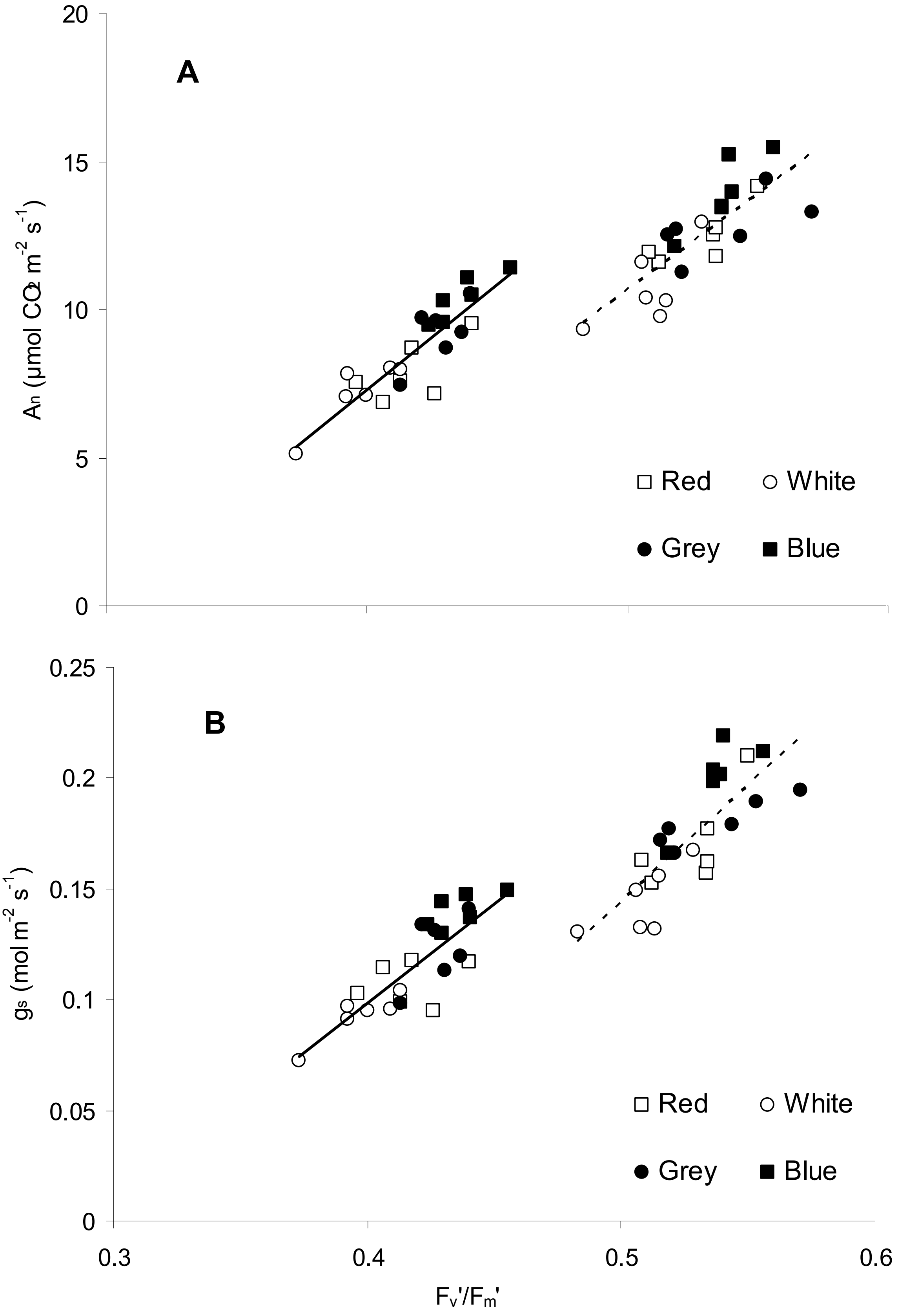

Fig. 3-9. Relationship between net photosynthetic rate, A_n, (A) and stomatal conductance, g_s, (B) with efficiency of light harvesting of PSII, F_v'/F_m' in 'Fuji' apple leaves grown under different colored shade nets during midmorning (dashed line) and midday (solid line). Fitting parameters of linear equation [(A_n or g_s) = a + b F_v'/F_m'] in (A) midmorning: a=-21.8, b=64.8 (r^2=0.63**); midday: a=-21.2, b=71.2 (r^2=0.78**); in (B) midmorning: a=-0.38, b=1.05 (r^2=0.64**); midday: a=-0.26, b= 0.9 (r^2=0.69**).

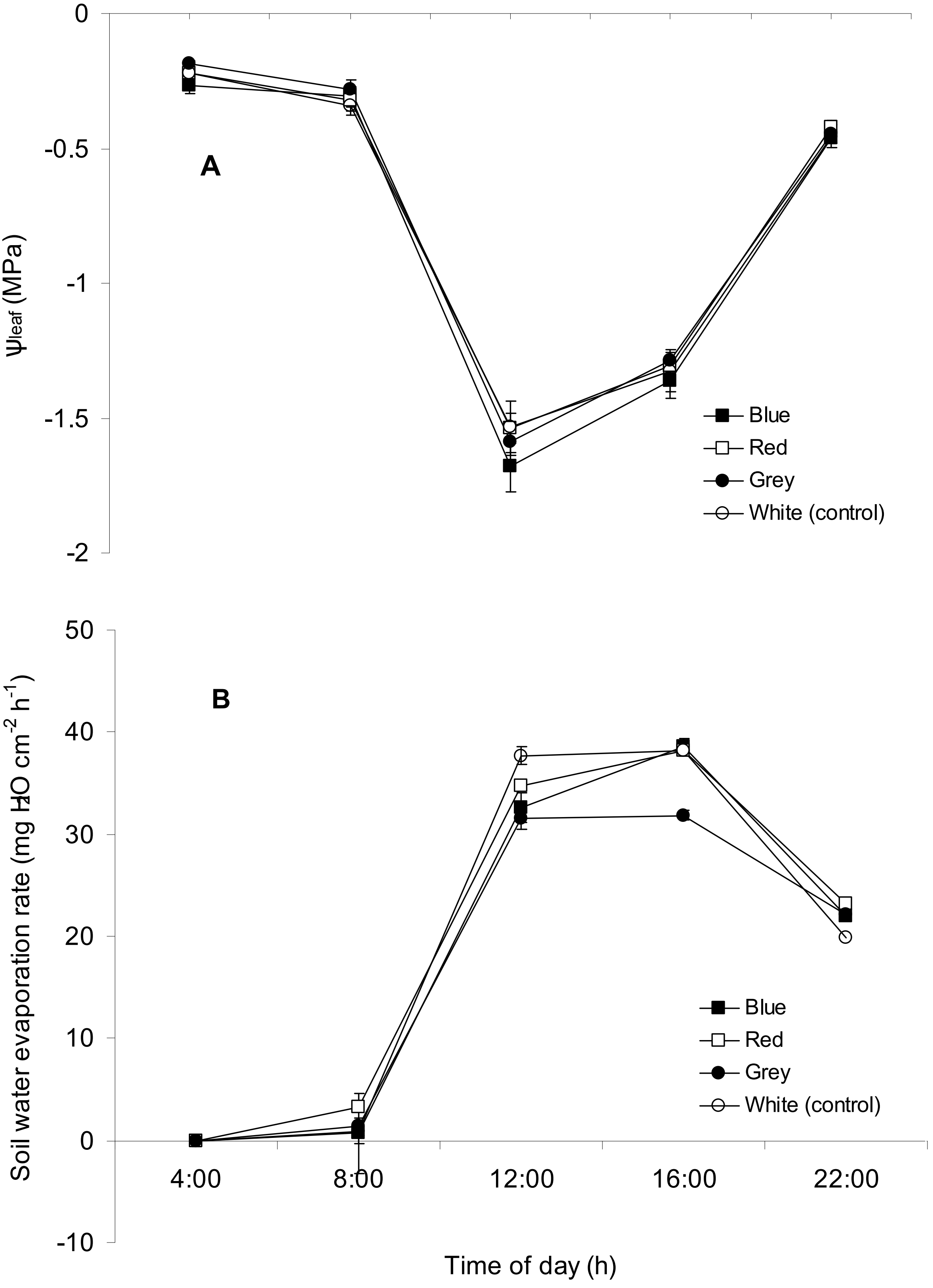

Fig. 3-10. The effect of colored shade nets on daily course of (A) leaf water potential, ψ_{leaf}, and (B) and soil water evaporation rate in 'Fuji' apple potted trees. (n= 5 – 10 ± SE). Measurements were taken on August 17, 2010.

Chapter IV

IS FRUIT GROWTH IN APPLES AFFECTED BY COLOR SHADE NETS? A STUDY APPROACH

INTRODUCTION

The effects of light conditions on apple fruit growth have been widely documented. Brief reduction of light availability by heavy shading during early stages of fruit growth reduce the apple fruit growth potential and induce fruit drop (Byers *et al.,* 1991; McArtney *et al.,* 2004; Zibordi *et al.,* 2009). Light conditions also affect fruit growth by affecting carbohydrate partitioning between fruit and shoots. Shoots exposed to full sunlight were able to export photo-assimilates to fruit 3 weeks after bloom, while similar export was reached by 70% shaded shoots only 5 weeks after bloom. Thus, early in the season, under sunlight limitation shoot growth has priority over the fruit for photo-assimilate allocation with detrimental effect on fruit growth potential (Tustin *et al.*, 1992; Corelli-Grappadelli *et al.* 1994; Bepete & Lakso 1998).

Implementation of net protection is gaining popularity in modern apple plantation around the world to protect orchards against excessive solar radiation damage, hailstorms and flying pests (Blanke, 2007). However, netting considerably affects the light availability which should in turn affect fruit growth potential. In South Africa, shade nets reduced fruit growth in ‘Royal Gala’ and ‘Cripp’s Pink’ which was associated to reduced net CO_2 assimilation (Gindaba & Wand, 2005). However, in the same fruit growing district, increased fruit growth was found under shade netting in ‘Fuji’ apples (Smit, 2007). In Australia, Middleton & McWaters (2002) showed that reduced fruit growth under hail nets was associated to vegetative vigor, while in weaker apple trees netting increased fruit size. Studies in Spain concluded, on the other hand, that anti-hail nets did not affect fruit size in ‘Mondial Gala’ (Iglesias & Alegre, 2006), showing that the effect of shade and/or hail nets on apple fruit growth is still a matter of discussion. Recently, colored nets have been developed as new technological tools in hope to improve fruit growth (Shahak *et al.,* 2008). The concept is that modification of light quality conditions (i.e. spectra light composition) could modify photosynthetic and morphogenetic processes involved in plant yield and quality (Shahak *et al.,* 2008). However, the potential advantage from the application of this technology on improving fruit growth in apple trees is still unclear. Recent reports showed that colored nets could be affecting fruit growth potential in apple orchards. However, the role of light quality in this response is still unclear, since effects were attributed more to changes in light availability (quantity of light) than quality (Solomakhin & Blanke, 2008). In early studies we demonstrated that colored shade nets affect differentially leaf gas exchange and photosynthetic capacity in apple trees (Bastías *et al.,* 2009) as well morphological development of shoots mediated by changed light quality conditions, specially in red, blue and NIR light spectra (*see Chapters II and III*). Net photosynthetic capacity and shoot growth strongly affect carbohydrate availability for fruit growth (Corelli-Grappadelli *et al.* 1994; Bepete & Lakso 1998; Wünsche *et al.,* 2005). The aim of the present research was to study the effect of colored shade nets with differential light transmission in blue, red and NIR spectra on apple fruit growth.

MATERIALS AND METHODS

Plant material and experimental design

The experiment was carried out at the University of Bologna Experiment Research Station (44°30'N; 10°36'E; 27 m a.s.l.) on three-year-old 'Fuji' apple trees grafted on dwarfing M9 rootstock planted in 40 l pots (1:2 sand and soil mix) and randomly assigned to three N-S oriented rows at 2.5 x 1.0 m spacing. At the end of pollination (April 29, 2010) Grey, Blue, Red colored shade nets (ChromatiNet®, Polysack Industries, Negev, Israel) with 40% nominal shade factor were placed on plots of 18 trees. White neutral net at 20% shading was included as control. Nets were placed over a metal tunnel arc 6 m wide and 3.5 m high; the East and West sides of the tunnel were covered to the ground, while the North and South ends of the tunnel were left uncovered to insure good air circulation. Water supply was managed by an automated drip irrigation system, while standard fertilization was applied at week intervals. The experiment was arranged in a completely random design, selecting from the four central trees per color for all assessments; the rest of the trees served as guards to avoid the influence of unfiltered light from outside.

Crop load establishment

At full bloom (April 21, 2010) 'Granny Smith' apple trees were placed in the alleyways for pollination. Since climate conditions were not totally favorable to adequate bee flight, hand pollination was carried out in all treatments. Receptive flowers were hand pollinated at least three times and until all flowers were opened. Once fruit set occurred, trees were hand thinned twice: 19 and 25 days after full bloom (DAFB) (May 10 - 16, 2010). At 19 DAFB, all lateral fruit per cluster were removed and only the king fruit was kept. At 25 DAFB all fruit per tree were counted and the crop load was adjusted to 5 fruit per cm^2 of trunk cross section area, TCSA

(Robinson, 2008). An attempt was made to leave fruit evenly spread in the tree, leaving between 1 and 2 fruit per branch.

Fruit and shoot growth rate

Five fruit per tree were selected and tagged; two were taken from terminal clusters and three from spur clusters. Maximum equatorial diameter (mm) per each fruit was periodically measured from 33 until 160 DAFB using a digital caliper. To convert the fruit diameter (D, mm) to fruit fresh weight (W, g) as was proposed by Palmer *et al.* (1997), on each sampling date twenty fruit (180 for whole season) were taken from adjacent trees and the following relationship between fruit diameter and fruit weight was obtained:

$$W = a \times D^b \tag{1}$$

where a and b were 0.0011 and 2.77, respectively and R^2=0.99

Absolute (AGR, g day^{-1}) growth rate was estimated (Zibordi *et al.,* 2009) as:

$$AGR = (Wt_1 - Wt_0) / (t_1 - t_0) \tag{2}$$

where Wt_1 and Wt_0 are the estimated fruit weight (g) at a given time (t_1) and at the previous sampling (t_0), respectively.

Simultaneously, five non fruiting bourse shoots per tree were randomly tagged and shoot length (cm) was measured at the same time of fruit measurements. Shoot absolute extension rate (AER, cm day^{-1}) was estimated (Grossman & DeJong, 1995) as:

$$AER = (SLt_1 - SLt_0) / (t_1 - t_0) \tag{3}$$

where SLt_1 and SLt_0 are shoot length (cm) at a given time (t_1) and at the previous sampling (t_0), respectively.

Finally, from 65 to 70 DAFB fruit diameter was measured at 15 min intervals by electronic custom-built gauges (Morandi *et al.,* 2007). The sensors were randomly placed on at least seven fruit per treatment. Data collection was made using a CR-1000 and CR100 data-loggers (Campbell Scientific Ltd., Leicestershire, UK) and calculation of hourly RGR (mg g^{-1} day^{-1}) was computed following the method proposed by Morandi *et al.* (2010) as AGR / Wt_0

Plant performance and fruit quality

At harvest, total yield (kg), fruit number and mean fruit weight (g), trunk cross section area (TCSA, cm^2), crop load (fruit cm^{-2} TCSA) and total shoot length (m) were recorded from four central trees. Leaf area per tree (m^2) was estimated weighing all the leaves. One hundred leaves per treatment were randomly collected, leaves were weighed and scanned and leaf area was measured by the Aequitas image analysis software (DDL Ltd, Cambridge, UK). Total leaf area per tree was estimated by a regression analysis between leaf weight and single leaf area. A sample of 40 fruit was used for fruit quality analysis. fruit were weighed and diameter (mm) was measured. Skin color was measured by quantification of L*, a*, b* chromaticity parameters in both exposed (reddest) and non exposed side (greenest) of each fruit using a Minolta Chroma Meter CR-200 portable tristimulus colorimeter (Minolta, Osaka, Japan). Color data were expressed as Hue angle (°) as was proposed by MacGuire (1992). Fruit maturity degree was measured by a non-destructive DA-Meter spectrometer and expressed as I_{AD} index (Ziosi *et al.,* 2008). Flesh firmness was quantified by a TR 53205digital firmness tester (Turoni, Forli, Italy). Sugar content was measured as soluble solid concentration (SSC, %) using a digital PR32 refractometer (Atago, Tokyo, Japan). Fruit dry matter concentration is a newly proposed quality metric for apples and could be used to predict fruit quality at postharvest (Palmer *et al.,* 2010), thus fruit dry matter (%) was measured taking a sample of 4 fruit per tree (16 fruit per treatment) and a sample of 20 gr. (including, skin, flesh and core) per each fruit was used to estimate dry matter by weighing it fresh and after reaching constant weight in a forced-draft oven at 60°C.

Statistical analysis

Statistical significance of colored shade nets were estimated by one- way ANOVA to test at $p<0.05$ and 0.001, respectively, followed by Student-Newman-Keuls test for multiple comparison of mean values. Regression analysis was used to estimate the relationships between variables. Statistical tests and fitting parameters of regression models were done by Statgraphics Centurion XVI software (StatPoint Technologies, Inc., Warrenton-Virginia, USA).

RESULTS

Fruit and shoot growth

Colored shade nets affected fruit fresh weight. During the whole season fruit under the Grey net were larger ($p<0.05$) than under the control (White net; fig. 4.1). Early in the season (33 – 47 DAFB) fruit under Grey net were 40 – 45% larger than control, but this difference was reduced through the growing season and reached only 25% at harvest. Although seasonal fruit weight variation under Blue net did not differ compared to White and Red nets, later in the season (from 131 DAFB), fruit under the Blue net presented fruit weight values slightly higher (non significant) than White and Red nets (Fig. 4-1). Absolute fruit growth rate (AGR) was highest under Grey net at 47 ($p<0.01$), 56 ($p<0.001$) and 83 ($p<0.05$) DAFB (Fig. 4-2 A). At 47 DAFB, AGR under Grey net was 0.3 g day^{-1} greater than those under White net, whereas at 83 DAFB its growth rate was 0.5 g day^{-1} higher than those grown under Red and White nets (Fig. 4-2 A). At 56 DAFB the AGR value did not differ among Grey and White nets, but it was 0.2 – 0.3 g day^{-1} greater than Red and Blue nets. In all treatments maximum AGR values were observed at 103 DAFB and these were highest in Grey and Blue nets (2.2 and 2.1 g day^{-1}, respectively). Shoot growth rate was similar among colored shade nets (Fig. 4-2B), but was higher under Blue net, with significant ($p<0.05$) differences at 41 DAFB, where the value of shoot absolute extension rate (AER) reached 0.28 cm day^{-1}, i.e. over 50% (0.15 cm day^{-1}) higher than those recorded under the Grey, Red and White nets (Fig. 4-2B). During the rest of the season there were no differences in AER among treatments, except during a second short burst of shoot growth at 103 DAFB, when AER under Blue net was also higher than the other treatments (Fig. 4-2B). A negative relationship ($p<0.01$) was found between AGR and AER, where 32% of the AGR differences among treatments were explained by changes in AER (Fig. 4-3).

Daily relative growth rate (RGR) measured between 65 – 70 DAFB did not differ among treatments (Fig. 4-4). However, it was observed that fruit grown under White net presented a more marked and prolonged period of shrinkage compared to the other net treatments. Negative values of RGR were observed from 10:00 to 18:00 in apples grown under White net, while in Blue and Grey nets minimal values of RGR were registered between 11:00 and 16:00. From 16:00 – 18:00 hours fruit under Blue and Grey nets grew more rapidly than those under Red and White nets (Fig. 4-5).

Plant performance and fruit quality

Mean fruit weight per tree under Blue and Grey nets was higher than the White net (219.9 and 211.4 g $fruit^{-1}$ *vs*. 187.8 g $fruit^{-1}$, respectively, $p<0.01$; table 4.1). Mean fruit weight did not differ under Red net compared to Grey net, or compared to White control net (Table 4-1). Fruit yield (kg $tree^{-1}$) was highest under the Blue net and up to 57% greater than control (White net). Total leaf area per tree was also highest under Blue net, with up to 0.5 m^2 more than Red, Grey and control White nets. There were no differences in crop load, total shoot length and TCSA parameters among net treatments (Table 4-1). Fruit quality parameters were also affected by colored shade nets (Table 4-2). Fruit diameters were consistent with fruit weight, thus significantly ($p<0.001$) larger fruit (~ 80 mm) were obtained under Blue and Grey nets, but they contained less sugar ($p<0.001$) compared to control (White net). Apple color on the exposed side of fruit was not significantly affected by colored shade nets ($p>0.05$), however fruit that grew under Blue net presented less color development in the shaded cheek. Flesh firmness was increased under Red net ($p<0.01$) and these values were up to 10% greater than those obtained under Blue and Grey nets. Fruit ripening stage estimated by I_{AD} index as well fruit dry matter (DM) concentration parameters did not differ among net treatments (Table 4-2).

DISCUSSION

Early fruit growth development and absolute growth rate (AGR) were highest under Grey net (Fig. 4-1 and 4-2A), thus 33 DAFB fruit under Grey net were 40 – 45% larger than control, while at final stages of fruit growth, both Blue and Grey nets presented more marked increases in fruit growth compared to control which was reflected in larger mean fruit size at harvest (Table 4-1). In previous experiments in this thesis, net CO_2 assimilation under Grey and Blue nets was found to be higher than Red and White nets (Table 4-3), confirming findings from the previous growing season, when non-fruiting apple trees were covered with colored shade nets (Bastías *et al.,* 2009). This suggests that during the first weeks after full bloom fruit growth under Blue and Grey nets was less limited by carbohydrate supply (Lakso *et al.,* 2001). On the contrary, the reduced net CO_2 assimilation observed under Red and White nets (Table 4-3) led to lower carbohydrate availability and increased competition between fruit and shoots. This reduction of photo-assimilates available to fruit (Forshey & Elving, 1989) explains the lower fruit weight (Table 4-1) and diameter (Table 4-2) at harvest.

The higher early AGR (from 41 – 56 DAFB) under Grey net could also be related to a net effect on air temperature. Warrington *et al.* (1999) found that apple fruit growth was very sensitive to temperature during early growth after bloom and they showed significant increases in fruit diameter by varying day/night temperature from 9/3 to 25/15 °C. Unfortunately, no data were collected about the diurnal and nocturnal temperature variations under colored shade nets, however previous studies in this dissertation (see *Chapter III*) showed that Grey net blocks an important proportion of NIR radiation transmission, which could increase night temperature by reducing the nocturnal heat losses and increase fruit growth rate. Furthermore, leaf gas exchange measurements under colored shade nets showed that reduction of NIR transmission under Grey net should also reduce daily heat stress (see *Chapter III*), although the information provided in this dissertation is not conclusive. At 103 DAFB Blue and Grey net reached maximum AGR values (~ 2 g day^{-1}), while under Red and White nets the trend was towards slower fruit growth (Fig. 4-2 A). Maximum AGRs are similar to those observed in 'Royal Gala' apples in New Zealand, between 2 - 2.5 g day^{-1}, which were associated to a marked effect of temperature increases during the cell division phase (until 60 DAFB) (Stanley *et al*., 2000). A significant effect of net color on AGR was also found later in the season (83 DAFB), i.e. after the time during which cell division in cortex is commonly considered complete (Denne, 1960; Pratt, 1988). Warrington *et al* (1999) observed that apple fruit growth rate is more sensitive to temperature early in the season, and less responsive during later fruit development. Calderón-Zavala *et al* (2004) found that early increases of day/night temperature of 33/28 °C effectively increased fruit growth rate, however when trees were exposed to the same temperature, although later in the season, strong reductions were observed in fruit growth. They proposed that under very warm conditions carbon might be less available for fruit growth. In this study, greater net CO_2 assimilation under Grey and Blue nets was closely linked to reduction of leaf temperature and VPD which led to increased leaf stomatal conductance (Table 4-3). Therefore, more abundant fruit growth observed under Blue and Grey nets can mainly be explained by greater carbohydrate availability. In fact, the microclimate conditions under Blue and Grey nets were less stressful, increasing stomatal conductance and photosynthetic capacity, therefore more availability of photo-assimilates for adequate cell division and fruit growth. Moreover, reduction of carbon availability, as observed under Red and White nets, affects the phloem loading in apples which could be reducing the hydrostatic pressure at the source end of phloem pathway, and therefore decrease the driving force for phloem translocation towards the fruit (Morandi *et al.,* 2011) and explain the reduction of AGR and final fruit size observed under Red and White control nets (Fig 4-2 A; Table 4-1 and 4-2). Apple fruit grow by continuous expansion during the night and shrinkage during the day (Tromp, 1984) and the amplitude of fruit shrinkage is strongly influenced by environmental conditions (radiation and VPD) that affect directly the water outflows by skin transpiration from fruit (Jones & Higgs, 1982) and/or back flow of water from fruit to other tree organs due mainly to excessive evaporative demand and reduced leaf water potential (Tromp, 1984; Lang, 1990). In this experiment, although fruit under the White net presented a more marked fruit shrinkage (Fig. 4-4), there were no differences in the fruit growth diurnal fluctuations among net treatments. Since fruit received more radiation (Table 4-3) and higher values of leaf-to-air vapor pressure deficit were registered under White net (*see Chapter III*), more shrinkage under White net could be associated to more transpiration from fruit and in line with

Jones & Higgs (1982). However, in previous studies a net effect on water soil conservation was also found (*see Chapter III*), thus Grey and Blue nets for example reduced soil water evaporation compared to control (White net). Furthermore, fruit under the White net presented greater soluble solid concentration (Table 4-2), which has normally been associated to water stress in apple trees (Lakso, 2003). Previous studies in this dissertation did not show (*see Chapter III*) differences in leaf water potential among colored shade nets, but this aspect should be more deeply investigated in future studies.

Apple growth is also strongly influenced by sink competition, including shoots and other fruit as has been widely documented (Forshey & Elving, 1989; Corelli-Grappadelli *et al.,* 1994; Wünsche *et al.,* 2005). In the present study crop load was standardized for all treatments and no differences in the crop load measured at harvest (Table 4-1) were observed , so no effects by fruit competition should be expected. However, shoot growth rate was highest under the Blue net (Fig.4.2B), therefore more competition among fruit and shoots may have occurred early in the season to the detriment of fruit (Forshey & Elving, 1989; Corelli-Grappadelli et al., 2004; Bepete & Lakso, 1998). These results can explain why fruit grown under the Blue net, which presents greater net CO_2 assimilation, presented lower AGR compared to Grey net and similar to control (Fig. 4-2A). Previous studies showed the importance of light conditions on apple shoot and fruit competition via carbohydrate supply, thus reduction of light availability by shading delayed the export of photo-assimilates from shoots to fruit and growing shoot tip has priority over the fruit for photo-assimilate allocation, early in the season when cell division rate determines the potential of fruit development (Tustin *et al*., 1992; Corelli-Grappadelli *et al*., 1994; Bepete & Lakso, 1998). In our study, all colored shade nets reduced light availability compared to the control, but also among the other nets, with the Grey reducing

PPFD more than the Blue net (Table 4-3). However, under the Blue net light quality conditions were widely changed compared to control, with a lower R:FR (600 – 700 nm: 700 – 800 nm), and an increased B:R ratio (400 – 500 nm: 600 – 700 nm) (Table 4-3). Lower R:FR values are associated to inactivation of plant phytochrome with increases in shoot elongation (Smith, 1982; Baraldi *et al.,* 1994) and could explain the early higher AER values under Blue net (Fig. 4-3). This effect is not clear cut, as high proportions of B:R are associated to shoot dwarfing via reduction of stem elongation (Rapparini *et al.,* 1999). In previous studies (see *Chapter II*) apple trees under Blue net presented a greater proportion of short shoots but with a greater shoot length overall. These effects of the Blue net on shoot growth could be a response to shade avoidance and/or phototropism, linked to changes in light quality combined to reduced PAR intensity (Franklin, 2008). A negative relationship was found between AER and AGR. Thus, changes in AGR under colored nets were partially (32%) but significantly influenced by changes in shoot growth (Fig. 4-3). Corelli-Grappadelli *et al* (1994) and Bepete & Lakso (1998) underlined the importance on light availability (i.e. light quantity) on shoot : fruit growth balance and carbohydrate partitioning in apple trees. In the present study we obtained an additional finding: light quality conditions also play an important role on shoot:fruit growth balance. In this direction technological improving in colored shade nets could help to obtain more successfully results in shoot:fruit growth balance management and thus to obtain more positive effect on fruit growth potential in apples.

CONCLUSIONS

Apple fruit growth was significantly affected by colored shade nets. The best results in final fruit size were obtained under Blue and Grey nets. Microclimate conditions under Grey and Blue were more favorable to increases in fruit growth potential in apples mainly through increased carbohydrate availability.

Colored shade nets affected also the early shoot: fruit growth balance mainly via modifications of light quality. Colored shade nets could be an interesting tool to shoot:fruit growth balance management and improve fruit growth potential in apples.

TABLES AND FIGURES

Table 4-1. The effect of colored shade nets on plant performance components in 'Fuji' apple trees.

Net treatment	Fruit yield (Kg)	Fruit weight (g)	TCSA (cm^2)	Crop load (n° cm^{-2} TCSA)	Leaf area (m^2)	Total shoot length (m)
Blue	5.5 a[1]	219.9 a	7.2	3.5	2.1 a	11.0
Grey	4.2 ab	211.4 ab	6.2	3.1	1.5 b	9.3
Red	4.2 ab	198.7 bc	6.1	3.4	1.7 b	8.5
White	3.5 b	187.8c	7.3	2.6	1.6 b	8.9
Net effect	*	**	NS	NS	*	NS

[1] Mean separation within columns by the Student-Newman-Keuls test. ^{NS}; *; **; ***: non significant and significant at $p < 0.05$, 0.01 and 0.001, respectively (n=4 trees).

Table 4-2. The effect of colored shade nets on fruit quality traits in 'Fuji' apple trees.

Net treatment	Diameter (mm)	Color (° HUE)		I_{AD}	SSC (%)	Firmness (kg cm^{-2})	DM (%)
		Exposed side	Shaded side				
Blue	80.8 a[1]	49.4	98.3b	1.0	15.0 d	8.4b	18.1
Grey	79.0 a	48.7	86.0 a	1.0	15.7 c	8.3 b	18.7
Red	75.1 b	49.8	93.6 ab	1.1	16.3 b	9.1 a	20.1
White	75.3 b	44.4	83.3 a	1.1	17.0 a	8.7 ab	19.1
Net effect	***	NS	**	NS	***	**	NS

[1] Mean separation within columns by the Student-Newman-Keuls test. ^{NS}; *; **; ***: non significant and significant at $p < 0.05$, 0.01 and 0.001, respectively (n=40 fruit).

Table 4-3. The effect of colored shade nets on leaf photosynthetic and plant morphogenetic parameters measured under field conditions (Adapted from *Chapter II and III*)

Parameters	Net treatments				*Net effect*
	White	Blue	Red	Grey	
PPFD ($\mu mol\ m^{-2}\ s^{-1}$)	1020.0 ± 13.5[1]	736.9 ± 15.7	745.2 ± 11.6	639.5 ± 7.6	***
A_n ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	7.1 ± 0.45	10.4 ± 0.31	7.9 ± 0.41	9.2 ± 0.42	***
g_s ($mol\ m^{-2}\ s^{-1}$)	0.10± 0.004	0.14± 0.003	0.11± 0.004	0.12± 0.006	***
R:FR ratio	0.960 ± 0.006	0.870 ± 0.004	0.930 ± 0.004	0.940 ± 0.010	***
B:R ratio	0.700 ± 0.006	0.920 ± 0.010	0.550 ± 0.007	0.720 ± 0.006	***

[1] Each value represents the mean ± SE. ^{NS}; *; **; ***: non significant and significant at $p < 0.05$ and 0.001, respectively.

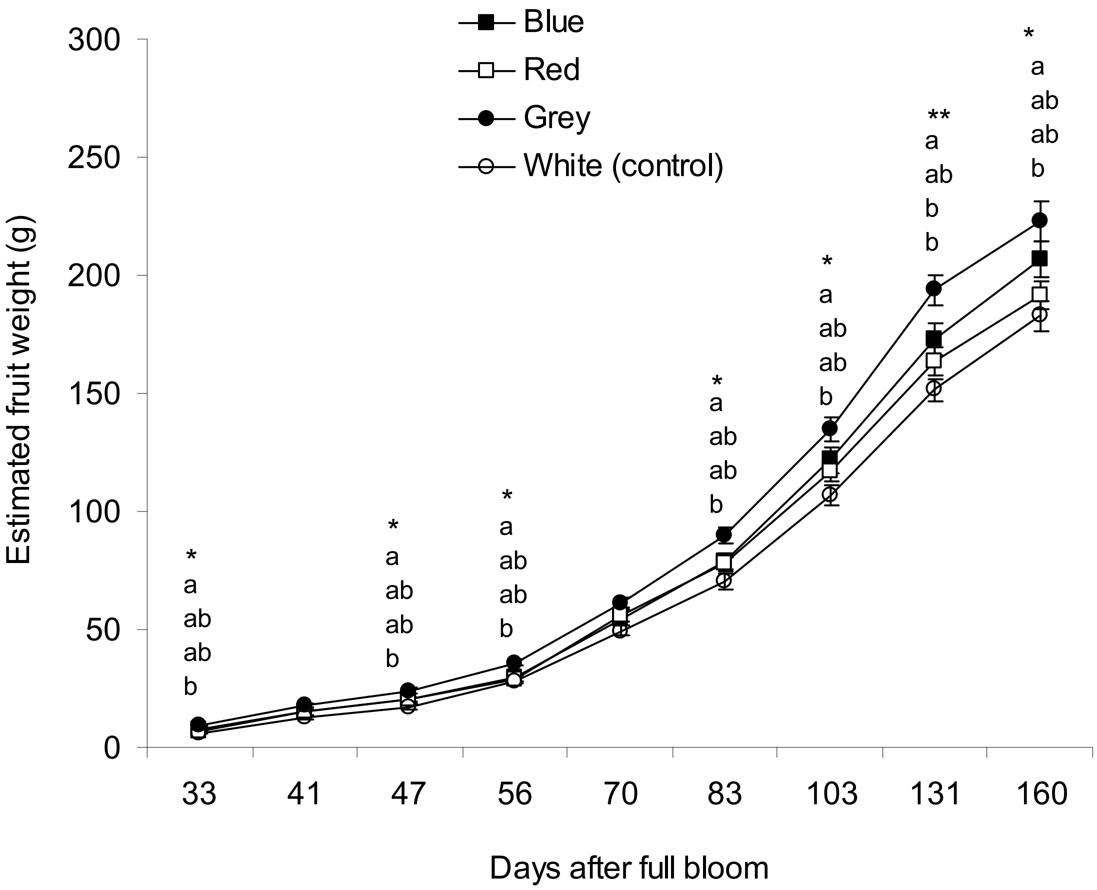

Fig. 4-1. The effect of colored shade nets on fruit growth in ‘Fuji’ apple trees. Each date represents the mean ± SE of 20 fruit or shoots. Mean separation within points by the Student-Newman-Keuls test *; **; ***: significant at $p < 0.05$, 0.01 and 0.001, respectively.

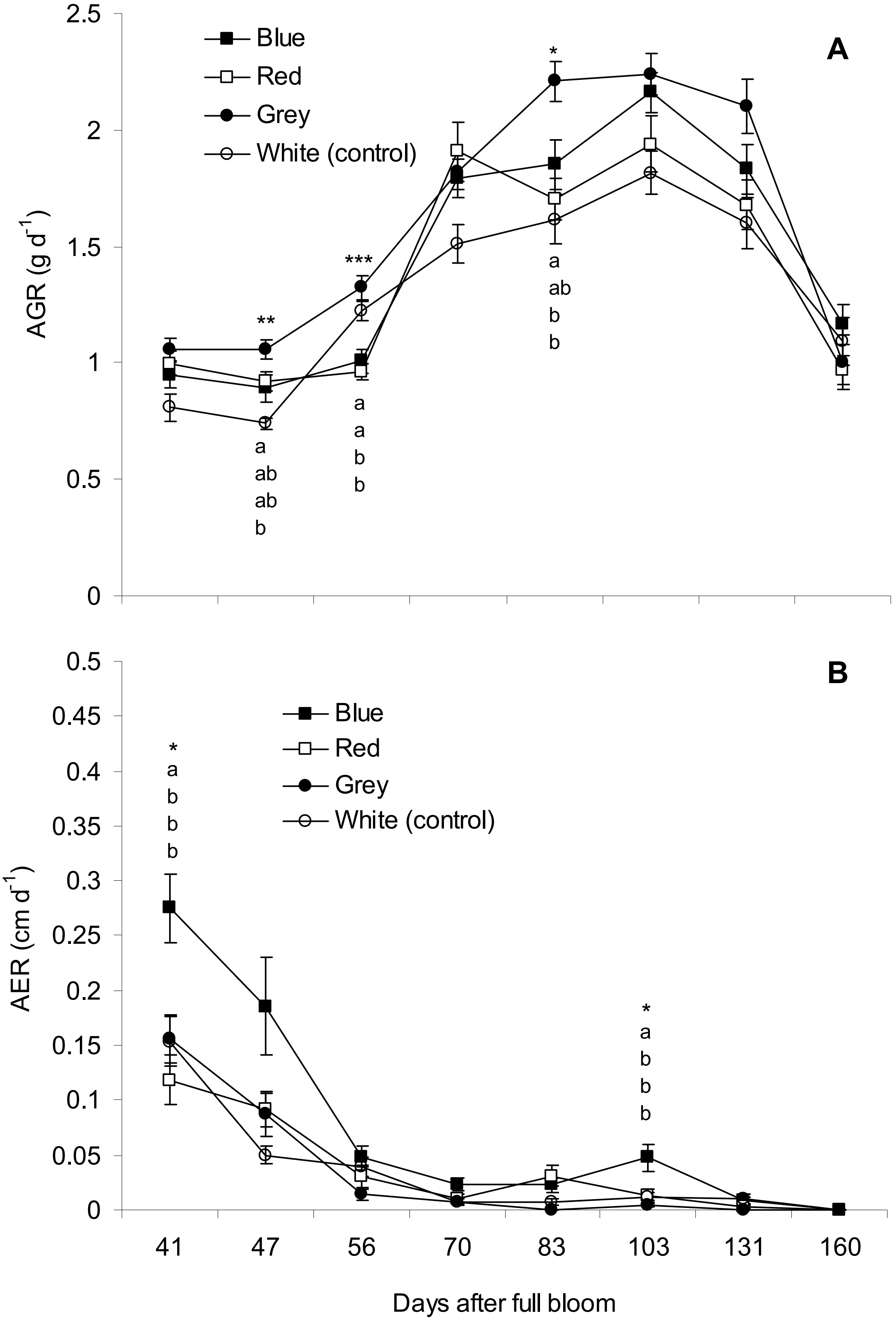

Fig. 4-2. The effect of colored shade nets on seasonal (A) absolute fruit growth rate, AGR, and (B) absolute shoot extension rate, AER, in ‘Fuji’ apple trees. Each date represents the mean ± SE of 20 fruit. Mean separation within points by the Student-Newman-Keuls test. *; **; ***: significant at $p < 0.05$, 0.01 and 0.001, respectively.

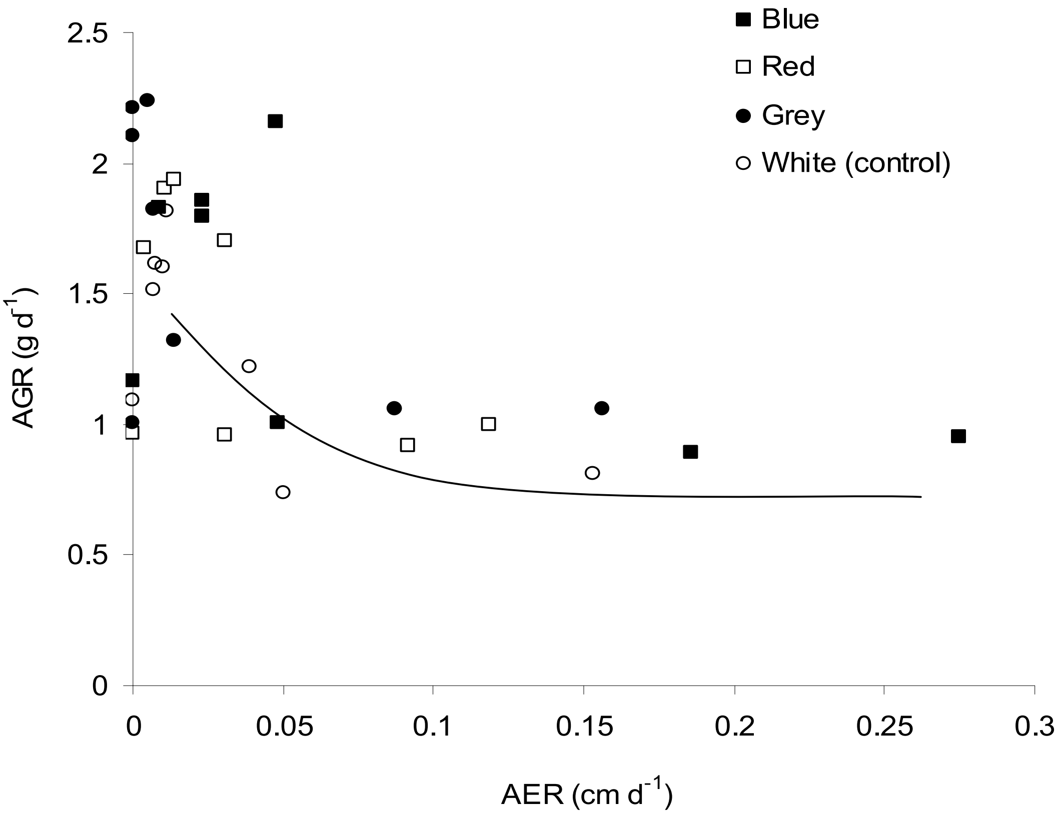

Fig. 4-3. Seasonal relationship between absolute fruit growth rate, AGR, and shoot absolute extension rate, AER in 'Fuji' apple trees grown under different colored shade nets. Regression equation is [AGR = 1/ (0.68 + 2.45AER)] (r^2=0.32**), significance at p<0.01. Each point represents the mean of 20 fruit or shoots.

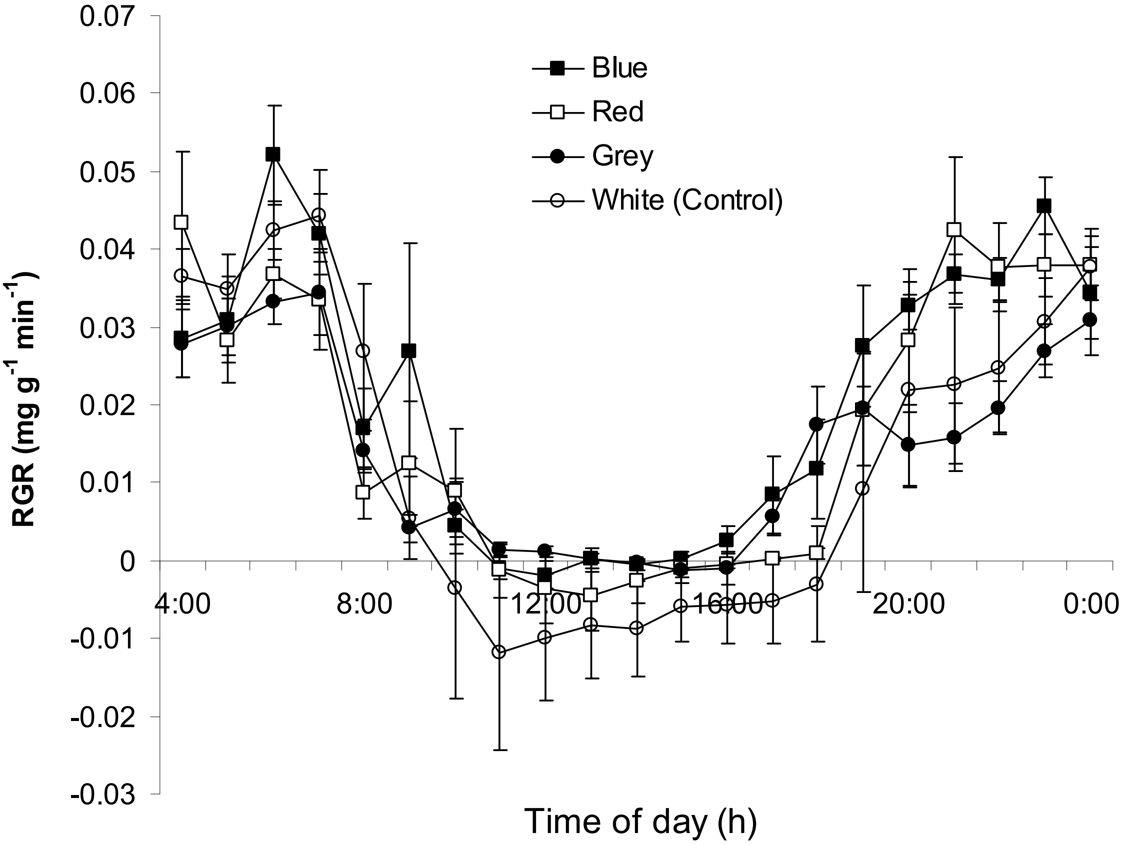

Fig. 4-4. The effect of colored shade nets on the diurnal pattern of relative fruit growth rate in 'Fuji' apples. Each data point represents the mean ± SE of 7 fruit.

Chapter V

GENERAL CONCLUSIONS

"Light quantity" management (i.e. maximizing the interception and distribution of photosynthetically active radiation, PAR) through cultural practices such as pruning, training systems and tree arrangement for several decades has been a key technological aspect to ensure adequate production and fruit quality in apple orchards (Jackson, 1980; Palmer, 1989; Corelli & Sansavini, 1989; Wagenmakers, 1996; Wünsche & Lakso, 2000; Bastías & Widmer, 2002; Corelli-Grappadelli, 2003). From the results of the present PhD dissertation an alternative and innovative approach to the management of sunlight in apple orchards emerges: Management of *"Light quality"* (i.e. spectral composition of sunlight) using photo-selective colored nets. Using a simple experimental model based on the standardization of external factors such as soil, microclimate, water, nutrition, tree vigor and fruit load, it was demonstrated that shading with Blue, Red and Grey colored nets with different light transmission in the blue (400 – 500 nm), red (600 – 700 nm) and near infrared (700 – 1100 nm) wavelengths produced a differential effect, depending on net color, on morphological and physiological aspects in apple trees.

Blue and Red nets with similar amount of light transmission in the PAR spectrum (400 - 700 nm) showed significant differences in the proportion of blue : red (B: R), red : far red (R: FR) ratios as well as in the amount of near-infrared (NIR) transmitted. Thus leaves grown under Blue net were larger with higher chlorophyll content and lower leaf dry mass per area unit (LMA) resembling "shade leaves", whereas those grown under the Red net were smaller, presented lower chlorophyll content and greater LMA, resembling "sun leaves". However, net CO_2 assimilation by leaves under Blue net was higher than under Red net, because light composition under the Blue net (higher proportion of blue light and lower proportion of red and infra-red light) favored greater stomatal conductance which led to increases in leaf photosynthesis and better leaf photochemical capacity of PSII was also observed under Blue net. Moreover, at whole plant level we found that the terminal shoot growth rate and vegetative development (number of shoots, leaf area and proportion of short shoots) were higher under the Blue net compared to Red net due to light interaction effects among B:R, R:FR ratios and reduction on PAR (shading). All these results show that in the future beneficial effects could be achieved by simply managing blue and red light composition with colored nets on over expression and/or suppression of desired and/or undesired morphological and physiological traits as observed in the present study. Combined effects of these types of nets and/or modification of optical properties of net materials in terms of light spectra transmission are interesting aspects to explore in further research.

Although leaves under colored shade nets showed anatomical characteristics that in theory might be limiting the leaf photosynthetic capacity (lower palisade thickness and reduced stomata density in relation to control), on contrary under Blue and Grey nets, leaf photosynthesis was higher than control, because photosynthesis in the latter leaves was more limited by stomatal conductance, which was confirmed by the greater intrinsic water use efficiency parameter (IWUE) observed.

On the other hand, shading intensity (40%) and thread design under Grey net were similar to Red and Blue nets, however the Grey net reduced in greater proportion the amount of PAR and also the amount of NIR radiation (thermal radiation). Reduction of radiative and thermal radiation by Grey net had positive effects on stomatal conductance, photosynthesis and water soil conserving, demonstrating an interesting potential for apple production under extreme conditions such as low water availability, excessive solar radiation and high temperatures which could be limiting the stomatal conductance and net CO_2 assimilation. Furthermore, water soil conserving properties of Grey net could help reduce the irrigation needs in orchards especially under drought conditions.

Reduced plant stress, more stomatal conductance, more CO_2 assimilation and more carbohydrate availability under Blue and Grey nets are conducive to increases in fruit size as observed at commercial harvest. The positive effect of Blue and Grey nets on apple fruit growth seems to be very auspicious, however further validation studies at commercial scale are required. It is not known, for example, what could be the response of fruit growth under less stressful conditions, such as colder climates and with lower incident radiation. On the other hand, although apple fruit under the Blue and Grey nets were larger, they had lower sugar content and firmness than those from Red and control nets, therefore more integrative fruit quality analyses are necessary. A major competition between shoot and fruit growth under Blue net was observed early in the season. In this context, the competition could be limiting the fruit potential for growth in Blue net and probably under conditions where carbohydrate availability is non limited in uncovered apple orchards or under different color nets. Therefore further research is required in this direction.

Finally, the results obtained in the present PhD dissertation clearly demonstrate that *"Light quality"* management through photo-selective colored nets could be an alternative technology to address many of the most important challenges of modern fruit growing, such as: the need for the efficient use of natural resources (water, soil and nutrients) the reduction of environmental impacts and the mitigation of possible negative effects of global climate change. Although the use of colored nets is already taking hold among fruit growers, further investigations are required, including validation studies in different agro-environmental conditions (species, cultivars, climate, soil and water availability), as well as the incorporation of improvements (optical properties, combined net colors) to existing technology.

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