

## EXPLORING THE POTENTIAL USE OF PHOTO-SELECTIVE NETS FOR FRUIT GROWTH REGULATION IN APPLE

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The effect of shading (i.e. reduction of sunlight availability) on fruit growth physiology has been widely studied in apple (*Malus domestica* Borkh.), but little knowledge exist about fruit growth responses to changes in the light spectrum. The aim of the present research was to study the effect of use of colored nets with differential sunlight transmission in the blue (B, 400-500 nm), red (R, 600-700 nm) and far-red (FR, 700-800 nm) spectra on apple fruit growth and physiological associated responses. Three year old 'Fuji' apple trees were covered with 40% photo-selective blue and red shade nets, 40% neutral grey shade net, and 20% neutral white net as control. Red and blue net reduced in the same proportion (27%) the photosynthetically active radiation with respect to control. However, blue net increased by 30% and reduced by 10% the B:R and R:FR the light relations, respectively. Maximal fruit growth rate under blue and grey nets was 15-20% greater than control. Fruit weight under blue net was 17% greater than control, but no significant differences in fruit weight were found among red net and control. Leaf photosynthesis and total leaf area under blue net were 28% and 30% higher than control, respectively; with ensuing positive effect on tree net C assimilation rate and total dry matter production. Results suggest that shifting the B, R, and FR light composition with photo-selective nets could be a useful tool to manipulate the photosynthetic and morphogenetic process regulating the carbohydrate availability for apple fruit growth.

**Key words:** Sunlight spectrum management, fruit size, photosynthesis, morphogenesis, *Malus domestica*.

Implementation of net protection is gaining popularity in modern apple (*Malus domestica* Borkh.) plantations around the world to protect orchards against excessive solar radiation damage, hailstorms, and flying pests (Blanke, 2007). In apple trees, the effects of shade and/or hail netting on fruit growth development has been reported, but with different responses depending on climate conditions and cultivars. In South Africa, for example, shade nets reduced the fruit growth in 'Royal Gala' and 'Cripp's Pink' apples (Gindaba and Wand, 2005), whereas in the same fruit growing district, increased fruit growth was found under shade netting in 'Fuji' (Smit, 2007). Studies in Spain concluded, on the other hand, that covering the apple orchard with nets did not affect fruit size in 'Mondial Gala' (Iglesias and Alegre, 2006). Although the effect of netting on apple fruit growth is still a matter of discussion, most of these studies agree that fruit growth responses to netting are related to the effects of nets on reduction in light availability (Gindaba and Wand, 2005). The effects of light conditions on apple fruit growth have been documented. Brief reduction of light intensity by heavy shading during early stages of fruit

growth reduces apple fruit growth rate and induces fruit drop (McArtney *et al.*, 2004), and it was closely related to decreases in photosynthesis and tree C assimilation (Zibordi *et al.*, 2009). Light availability also affects fruit growth by affecting carbohydrate partitioning between fruit and shoots. Shoots exposed to full sunlight were able to export photo-assimilates to fruit 3 wk after bloom, while similar export was reached by 70% shaded shoots only 5 wk after bloom (Corelli-Grappadelli, 2003). 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 (Bepete and Lakso, 1998).

Recently and thanks to technological advances with photo-selective plastic filters, colored nets have been developed, which provide differential filtration of solar radiation together with physical protection, modifying widely the light conditions, but mainly the spectral light composition (Shahak *et al.*, 2008). In plants, including some fruit trees, it has been demonstrated that changes in light composition in red (R, 600-700 nm) far-red (FR, 700-800 nm), and blue (B, 400-500 nm) spectra modulate differentially morphological and photosynthetic responses. Lower proportion of R:FR, for example, increased shoot growth in peach trees, which is a typical phytochrome-mediated response (Baraldi *et al.*, 1994), while, still in peach, the higher proportion of B in relation to R light induces

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shoot dwarfing via reduction of stem elongation, a process mediated by the cryptochrome (Rapparini *et al.*, 1999). Moreover, in herbaceous plants, B and R light composition might affect the stomatal conductance, photosystem II efficiency and therefore leaf photosynthesis rate (Shimazaki *et al.*, 2007). Since changes in shoot growth and photosynthesis strongly affect carbohydrate availability for apple fruit growth (Bepete and Lakso, 1998; Zibordi *et al.*, 2009), variation in spectral light composition by photo-selective colored nets should differentially affect the fruit growth in apple trees. However, the potential use of this technology for apple tree and fruit growth regulation, as well as the physiological aspects involved, are still unclear, because very little is known regarding the effects of spectral light composition on plant physiological response in apple trees (Corelli-Grappadelli, 2003). The aim of the present research was to evaluate the effect of colored shade nets with differential light transmission in the blue, red and far-red spectra on the following aspects: i) seasonal and diurnal fruit growth rate; ii) vegetative growth and dry matter production; iii) photosynthesis and gas exchange; and iv) light intensity and light spectral composition.

## 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., Italy) on 3-yr-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 × 1.0 m spacing. At the end of pollination (29 April 2010) grey, blue, and red colored shade nets (ChromatiNet®, Polysack Industries, Negev, Israel) with 40% nominal shade factor were placed on plots of 18 trees. 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. Thus, in comparison to control, grey net was used to examine the effect of reduction of light quantity (neutral shade) and red and blue nets to evaluate the effect of changes in red, blue and far-red light composition (photo-selective shade). Nets were placed over a metal tunnel arc 6 m wide and 3.5 m high, and 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. 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 four trees of the central row per each treatment 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 (21 April 2010) 'Granny Smith' potted apple trees were placed in the alleyways for pollination. Since climate conditions were not totally favorable for 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 d after full bloom (DAFB) (10-16 May 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<sup>2</sup> 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 growth

Five fruits per tree (20 per treatment) 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), on each sampling date 20 fruit (180 for the whole season) were taken from adjacent trees and the following relationship between fruit diameter and fruit weight was obtained:  $W = a \times D^b$ ; where a and b were 0.0011 and 2.77, respectively and  $R^2 = 0.99$ . Absolute fruit growth rate (AGR, g d<sup>-1</sup>) was estimated by the following relation (Zibordi *et al.*, 2009):  $AGR = (W_{t_1} - W_{t_0}) / (t_1 - t_0)$ ; where  $W_{t_1}$  and  $W_{t_0}$  are the estimated fruit weight (g) at a given time ( $t_1$ ) and at the previous sampling ( $t_0$ ), respectively. From 65 to 70 DAFB fruit diameter variation 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 CR-1000 and CR100 data-loggers (Campbell Scientific Ltd., Leicestershire, UK) and daily relative fruit growth rate (RGR, mg g<sup>-1</sup> min<sup>-1</sup>) was computed as  $AGR/W_{t_0}$  (Zibordi *et al.*, 2009). At harvest, total fruit number, mean fruit weight (g), and trunk cross section area (TCSA, cm<sup>2</sup>) were recorded.

### Vegetative growth and dry matter production

Five non fruiting bourse shoots per tree (20 per treatment) were selected and shoot length (cm) was measured at the same time of fruit diameter measurements. Shoot absolute extension rate (AER, cm d<sup>-1</sup>) was estimated (Grossman and DeJong, 1995) as:  $AER = (SL_{t_1} - SL_{t_0}) / (t_1 - t_0)$ ; where  $SL_{t_1}$  and  $SL_{t_0}$  are shoot length (cm) at a given time ( $t_1$ ) and at the previous sampling ( $t_0$ ), respectively. When shoot growth ceased, total shoot length (m) was estimated over a total of four trees per color net. Before leaf fall (October 2010), four trees were dissected to leaves, annual shoots and fruits and total fresh weight was separately measured. Dry matter (%) was estimated taking samples

of four replicates of 50-100 g of tissue (leaf, fruit, and shoot) per each tree, which was weighted in fresh and after reaching constant weight in a forced-draft oven at 60 °C. 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), and total leaf area per tree was estimated by a regression analysis between total leaf weight and single leaf area.

### Photosynthesis and gas exchange

Leaf net photosynthesis rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), stomatal conductance ( $\text{mol m}^{-2} \text{ s}^{-1}$ ), and transpiration rate ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) were measured using a LI-6400 gas infrared gas analyzer (LI-COR, Lincoln, Nebraska, USA) at controlled and saturated photosynthetic photon flux density ( $1250 \mu\text{mol m}^{-2} \text{ s}^{-1}$  PPFD) provided by internal red/blue LED light. The saturation PPFD value was set as the average of PPFD measured under colored nets at the level of well illuminated leaves. All measurements were made on six well illuminated leaves per treatment, selected in the middle part of annual shoots. The assessments were carried out during a clear summer day (23 June 2010) and repeated during two times: 09:30 and 11:30 h, when maximum stomatal conductances are registered in apple trees (Giuliani *et al.*, 1997). Tree net carbon exchange rate (NCER,  $\mu\text{mol CO}_2 \text{ tree}^{-1} \text{ s}^{-1}$ ) was estimated as:  $\text{NCER} = \text{LNCER} \times \text{LA}$ ; where LNCER is the mean leaf photosynthesis rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) per tree and LA is the total leaf area per tree ( $\text{m}^2$ ).

### Light conditions

During three times (morning, midday, and afternoon) of a clear summer day, photosynthetic photon flux density (PPFD,  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) was measured by a QSO-S quantum sensor (Decagon Devices, Pullman, Washington, 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 a LI-1800 spectroradiometer (LI-COR, Lincoln, Nebraska, USA). Both sensors (quantum and optical fiber) were placed horizontally at 1 m above ground in the alleyway among the potted trees under full light conditions. The R:FR and B:R wavelength ratios were estimated as  $(600\text{-}700 \text{ nm})/(700\text{-}800 \text{ nm})$  and  $(400\text{-}500 \text{ nm})/(600\text{-}700 \text{ nm})$  respectively, according to Kittas *et al.* (1999). Readings were replicated in four points for each color net.

### Statistical analysis

Statistical significance of the effect of colored shade nets was estimated by one-way ANOVA  $P < 0.05$ , 0.01, and 0.001, respectively, followed by Student-Newman-Keuls test for multiple comparison of mean values. Statistical tests were done by Statgraphics Centurion XVI software (StatPoint Technologies, Inc., Warrenton, Virginia, USA).

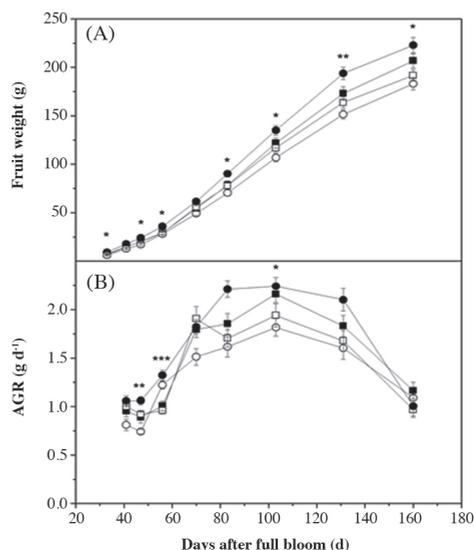
## RESULTS

### Light conditions

All colored shade nets reduced PPFD compared to control (Table 1). Red and blue net reduced PPFD in the same proportion (27%) with respect to control; while under grey net the reduction of PPFD was the highest (37%). Daily mean R:FR and B:R values did not change among neutral nets (white and grey), however daily mean R:FR under blue net was near 10% lower than other treatments (Table 1). Blue net increased by 30% the B:R ratio, while the red net reduced this proportion up to 20%, compared to white and grey nets (Table 1).

### Fruit growth

During the whole season fruit under the grey net were larger than under the white net (Figure 1A). 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



\*, \*\*, \*\*\*Significant at  $P < 0.05$ , 0.01, and 0.001, respectively.

**Figure 1.** Seasonal variation on (A) fruit growth and (B) fruit absolute growth rate (AGR) in 'Fuji' apple trees grown under blue (■), red (□), grey (●), and white (○) nets. Each data point represents the mean  $\pm$  SE of 20 fruit.

**Table 1.** Daily mean of light intensity and spectral light composition parameters estimated under colored shade nets.

Net treatments	PPFD ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	Wavelength ratios	
		R:FR	B:R
White (control)	1020.0a <sup>1</sup>	0.96a	0.70b
Red	745.2b	0.93b	0.55c
Blue	736.9b	0.87c	0.92a
Grey	639.5c	0.94ab	0.72b
Significance	***	***	***

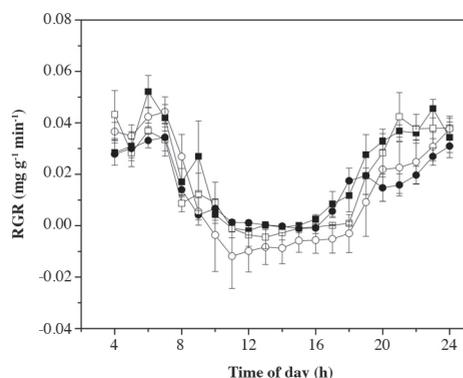
PPFD: photosynthetic photon flux density; R:FR = red:far-red estimated as  $(600\text{-}700 \text{ nm})/(700\text{-}800 \text{ nm})$ ; B:R = blue:red estimated as  $(400\text{-}500 \text{ nm})/(600\text{-}700 \text{ nm})$ .

<sup>1</sup>Mean separation within rows by the Student-Newman-Keuls test; n = 4 replicates. \*\*\* $P < 0.001$ .

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 than white and red nets (Figure 1A). The AGR value was highest under grey net at 47, 56, and 83 DAFB (Figure 1B). At 47 DAFB, AGR under grey net was 0.3 g d<sup>-1</sup> greater than those under white net, whereas at 83 DAFB its growth rate was 0.5 g d<sup>-1</sup> higher than those grown under red and white nets (Figure 1B). At 56 DAFB the AGR value did not differ among grey and white nets, but it was 0.2-0.3 g d<sup>-1</sup> greater than red and blue nets. In all treatments maximum AGR values were observed at 103 DAFB (Figure 1B) and under blue and grey nets these were near 15-20% (2.1 and 2.2 g d<sup>-1</sup>, respectively) greater than red and white nets (1.9 and 1.8 g d<sup>-1</sup>, respectively). Daily relative growth rate (RGR) measured between 65-70 DAFB did not differ among treatments (Figure 2). 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 h in apples grown under white net, while in blue and grey nets minimal values of RGR were registered between 11:00 and 16:00 h. From 16:00-18:00 h fruit under blue and grey nets grew more rapidly than those under red and white nets (Figure 2). At harvest, mean fruit weight under grey and blue nets was 12-17% higher than the white net (211.4 and 219.9 g fruit<sup>-1</sup> vs. 187.8 g fruit<sup>-1</sup>, respectively; Table 2). Mean fruit weight did not differ under red net compared to grey net, or compared to white net. However, the mean weight under blue net was 11% higher than red net. Crop load parameters such number of fruits per TCSA or per total leaf area, were not significantly affected by colored nets (Table 2).

### Vegetative growth, dry matter production, photosynthesis and gas exchange

Shoot growth rate was similar among colored shade nets (Figure 3), but was higher under blue net, with significant differences at 41 DAFB, when the value of AER reached



Each data point represents the mean ± SE of 7 fruit.

Figure 2. Diurnal pattern of fruit relative growth rate (RGR) in 'Fuji' apple trees grown under blue (■), red (□), grey (●), and white (○) nets.

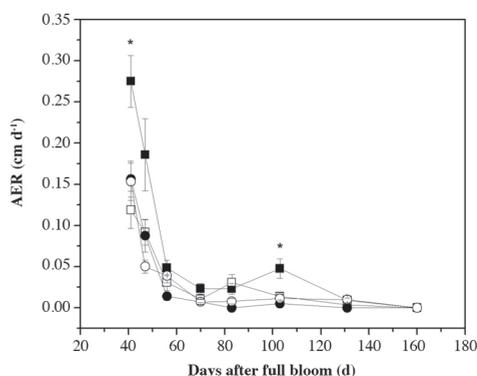
Table 2. Final fruit weight and crop load parameters estimated in 'Fuji' apple trees grown under colored shade nets.

Net treatment	Fruit weight	Fruits/TCSA	Fruits/Leaf area
	g	N° cm <sup>2</sup>	N° m <sup>2</sup>
Blue	219.9a <sup>1</sup>	3.5	12.0
Grey	211.4ab	3.1	13.0
Red	198.7bc	3.4	12.1
White (control)	187.8c	2.6	11.1
Significance	**	NS	NS

<sup>1</sup>Mean separation within rows by the Student-Newman-Keuls test; n = 4 trees; \*P < 0.05; \*\*P < 0.01; NS: non significant (P > 0.05); TCSA: trunk cross section area.

0.28 cm d<sup>-1</sup>, i.e. over 50% (0.15 cm d<sup>-1</sup>) higher than those recorded under the grey, red and white nets (Figure 3). 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 (Figure 3). Total leaf area per tree was also highest under blue net, with up to 0.5 m<sup>2</sup> (30%) more than red, grey and white nets (Table 3). Although total shoot length per tree did not differ among colored nets, total fresh weight of annual shoots under blue net was 58% greater than grey net (Table 3).

Total DM production (fruit + leaf + annual shoot) under blue net was on average 30% higher than red, grey and white nets (Figure 4A). Blue net presented the highest values of fruit, leaf and shoot DM compared with the other colored nets (Figures 4B, 4C, and 4D). Although fruit DM did not differ between blue and red nets, under blue net it was 30-50% greater than grey and white nets (Figure 4B). Dry matter production in vegetative structures (shoots +



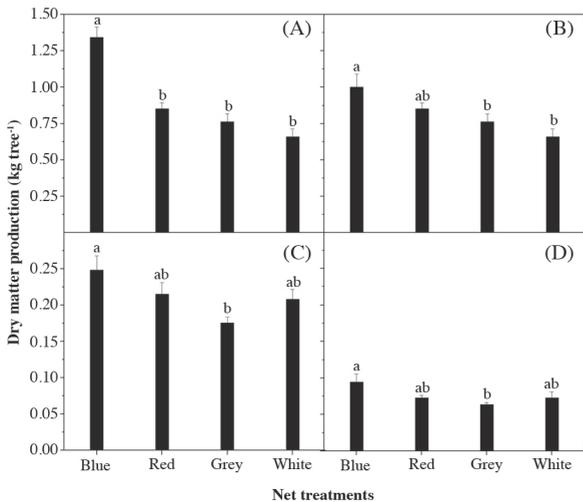
\*Significant at P < 0.05. Each data point represents the mean ± SE of 20 shoots.

Figure 3. Seasonal variation on shoot absolute extension rate (AER) in 'Fuji' apple trees grown under blue (■), red (□), grey (●) and white (○) nets.

Table 3. Vegetative growth responses of 'Fuji' apple trees grown under colored shade nets.

Net treatment	Total leaf area	Total shoot length	Total shoot fresh weight
	m <sup>2</sup> tree <sup>-1</sup>	m tree <sup>-1</sup>	kg tree <sup>-1</sup>
Blue	2.1a <sup>1</sup>	11.0	0.19a
Grey	1.5b	9.3	0.12b
Red	1.7b	8.5	0.15ab
White (control)	1.6b	8.9	0.14ab
Significance	*	NS	*

<sup>1</sup>Mean separation within rows by the Student-Newman-Keuls test; n = 4 trees; \*P < 0.05; NS: non significant (P > 0.05).



Each bar represents the mean  $\pm$  SE of four plants. Mean separation between bars by the Student-Newman-Keuls test.

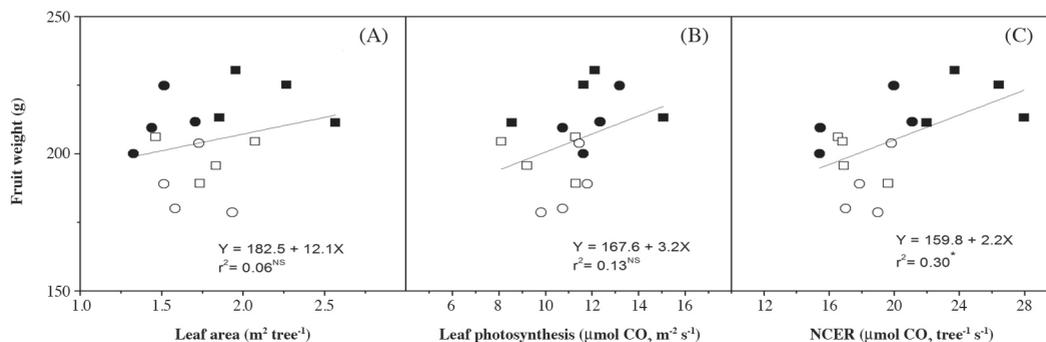
**Figure 4.** Total dry matter production of fruits + leaves + shoots (A), fruits (B), leaves (C) and shoots (D) in 'Fuji' apple trees grown under blue, red, grey, and white nets.

leaves) did not significantly differ among blue, red, and white nets. However, blue net increased shoot and leaf DM by 40-50% compared to grey net (Figures 4C and 4D). Leaf net photosynthesis was also increased by blue net. Net photosynthesis under blue net was 20-28% ( $2-3 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) higher than red and white nets, respectively (Table 4). The estimated value of NCER of trees grown under blue net was 40% ( $7 \mu\text{mol CO}_2 \text{ tree}^{-1} \text{ s}^{-1}$ ) greater

**Table 4.** Photosynthetic and gas exchange responses of 'Fuji' apple trees grown under colored shade nets.

Net treatment	Net photosynthesis $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	Stomatal conductance $\text{mol m}^{-2} \text{ s}^{-1}$	Transpiration $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$	NCER $\mu\text{mol CO}_2 \text{ tree}^{-1} \text{ s}^{-1}$
Blue	13.6a <sup>1</sup>	0.18	4.0a	25.0a
Grey	12.7ab	0.18	3.4ab	17.9b
Red	11.3b	0.16	3.3ab	17.4b
White (control)	10.6b	0.15	3.0b	18.4b
Significance	*	NS	*	**

<sup>1</sup>Mean separation within rows by the Student-Newman-Keuls test; n = 6 leaves and/or 4 trees; \* $P < 0.05$ ; \*\* $P < 0.01$ ; NS: non significant ( $P > 0.05$ ); NCER: Estimated whole tree net carbon exchange rate.



\*Significant at  $P < 0.05$ , <sup>NS</sup> non significant.

**Figure 5.** The response of fruit weight to: (A) Leaf area per tree; (B) leaf photosynthesis and (C) estimated whole tree net carbon exchange rate (NCER), in 'Fuji' apple trees grown under blue (■), red (□), grey (●) and white (○) nets.

than other net treatments. There were no differences in leaf photosynthesis among blue and grey nets. Although leaf stomatal conductance was not significantly affected by colored nets, transpiration rate of leaves grown under blue net was up to  $1 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  (33%) greater than control (Table 4). Regression analysis demonstrated that final fruit weight was poorly and not significantly related to leaf area and leaf photosynthesis variations. However a positive and significant relationship was found between fruit weight and tree NCER (Figure 5).

## DISCUSSION

The larger mean fruit size obtained at harvest under blue and grey net compared to control (white net) can be explained by the higher AGR values observed under grey and blue nets during the whole season. We found that maximum values of AGR under grey and blue nets were 20% greater than control (Figure 1B), and similar to those observed by Stanley *et al.* (2000) in 'Royal Gala' apples in New Zealand, between  $2-2.5 \text{ g d}^{-1}$ , who also demonstrated a positive relation between AGR increment and final fruit size. Apple fruit growth rate is affected by availability of photo-assimilates for adequate cell division (McArtney *et al.*, 2004; Zibordi *et al.*, 2009), while more prolonged cell division phase normally leads to higher values of AGR in apples (Stanley *et al.*, 2000). In our study, leaf photosynthesis under blue net was 28% higher than white net (Table 4), probably by a direct effect of PPFD reduction by shading due to leading to less stressful microclimate conditions and thus leading to greater net  $\text{CO}_2$  assimilation (Smit, 2007) and therefore more carbohydrate availability for adequate fruit growth. However, even though blue and red nets reduced by the same proportion the PPFD intensity (Table 1), they still displayed a differential effect on final fruit size. Under blue net fruit weight was 11% greater than red net (Table 2). This response could be explained by a spectral effect of light on leaf photosynthetic performance and the ensuing carbohydrate availability to fruit. Under blue net, B:R light proportion was 30% higher than red net (Table 1).

Leaf photosynthesis could be enhanced by an increase in blue light either via the stimulation of stomata opening and/or the improvement of Photosystem II efficiency (Matsuda *et al.*, 2004; Shimazaki *et al.*, 2007). In the present study, no differences in stomatal conductance were found (Table 4), probably because all measurements were made under controlled PPFD conditions and the  $VPD_{leaf-air}$  (2.2 kPa) value was not limiting leaf stomatal conductance (Dragoni *et al.*, 2004). Nevertheless, stomatal conductance tended to be higher under blue net (Table 4) and evaluation under ambient light environments demonstrated that blue net effectively increased the midday leaf stomatal conductance when compared to red net, while the efficiency of Photosystem II was also incremented (unpublished data). Therefore, the larger fruit growth observed under blue net compared to red net could perfectly be explained by the effect of more blue light proportion on the enhancement of stomatal and non-stomatal performance of leaf photosynthesis and consequently in photo-assimilate availability to fruit growth. However, it was not totally clear why final fruit weight and AGR were highest under grey net (Figure 1; Table 2), even when no significant differences in leaf photosynthesis were found among grey and white net (Table 3). In this context, Warrington *et al.* (1999) found that apple fruit growth is very sensitive to increased temperatures 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 showed that grey net blocks an important proportion of IR radiation transmission (Shahak *et al.*, 2008), which could increase night temperature by reducing the nocturnal heat losses and increase fruit growth rate. Furthermore, grey net was more effective in reducing PPFD compared to other colored nets (Table 1), which combined to reduction of IR radiation should be more effective to mitigate excessive heat stress. In fact, in the same experiment it was detected by open gas exchange analysis system that leaf temperature under grey net was 1-2 °C lower than control (unpublished data). Since C might be less available for apple fruit growth (Calderón-Zavala *et al.*, 2004) under very warm conditions, reduction of heat stress by grey net could increase carbohydrate availability for fruit growth.

On the other hand, apple fruits grow by continuous expansion during the night and shrinkage during the day and the amplitude of fruit shrinkage is strongly influenced by environmental conditions (radiation and vapor pressure deficit) that affect directly the water outflows by fruit skin transpiration from and/or back flow of water from fruit to other tree organs (Morandi *et al.*, 2011). In this experiment, although there were no differences in the fruit growth diurnal fluctuations among

net treatments, fruit under the white net presented a more marked daily fruit shrinkage (more negative RGR values) compared to blue and grey nets (Figure 2). The PPFD under white net was between 27 to 37% higher than blue and grey nets, respectively (Table 1), which should normally lead to higher leaf-to-air vapor pressure deficit in apple orchards (Smit, 2007), explaining, at least in part, the tendency to more fruit shrinkage under white compared to grey and blue nets. Moreover, increases of C availability affect phloem loading in apples, which could be increasing the hydrostatic pressure at the source end of phloem pathway, and therefore increase the driving force for phloem translocation towards the fruit (Morandi *et al.*, 2011) and could explain the tendency to higher RGR values registered under blue net compared to control (Figure 2).

Apple growth is also strongly influenced by sink competition, including shoots and other fruit as has been widely documented (Corelli-Grappadelli, 2003; Wünsche and Ferguson, 2005). In the present study crop load was standardized for all treatments and no differences in the final crop load parameters were observed (Table 2), so no effects by fruit competition should be expected. Under shading conditions that reduced sunlight availability, shoot growth has priority over the fruit for photo-assimilate allocation with detrimental effect on fruit growth potential (Bepete and Lakso, 1998). In our study, a reduction between 27%-37% of PPFD under red and grey shade nets (Table 1) did not alter shoot growth and therefore this growth should not have been limiting carbohydrate allocation to fruit. However, shoot growth rate was highest under the blue net (Figure 3), due to significant reduction of R:FR values (Table 1), as was observed under blue net, and normally associated to inactivation of plant phytochrome with increases in shoot elongation (Smith, 2000). This effect is not clear cut, as high proportions of blue light under blue net should be associated to shoot dwarfing via reduction of stem elongation (Rapparini *et al.*, 1999). However, the stimulation of shoot growth rate by reduction of R:FR ratio can explain, in part, why fruit grown under the blue net, which shows greater net CO<sub>2</sub> assimilation (Table 4), presented at early season lower AGR compared to grey net (Figure 1B), since more competition for carbohydrate availability among shoot and fruit in early stages of development should be expected (Bepete and Lakso, 1998; Corelli-Grappadelli, 2003). Moreover, although blue and red nets received similar PPFD (Table 1), total leaf area per tree was significantly incremented by blue net (Table 3), which could be explained by the direct effect of blue light on phototropic responses such as stimulation of leaf expansion under ambient conditions with reduced PPFD combined with a high proportion of blue light (Takemiya *et al.*, 2005). Since leaves were well illuminated inside the tree canopies, greater leaf area per tree under blue

net (Table 3) might be improving the efficiency in light intercepted for photosynthesis (Wünsche and Lakso, 2000). This assumption was confirmed with the estimated values of tree NCER, which demonstrated that net C assimilation rate by trees grown under blue net was 40% highest (Table 4); and explains the highest total DM production observed under blue net (Figure 3). Furthermore, a significant and positive relationship was found between fruit weight and tree NCER, but not when was separately related to leaf area and leaf photosynthesis (Figure 5). Apple tree NCER is strongly influenced by single leaf photosynthesis capacity and total leaf area exposed to sunlight (Giuliani *et al.*, 1997), while the carbohydrate availability for fruit growth by sink:source relationship among vegetative and reproductive organs (Bepete and Lakso, 1998; Corelli-Grappadelli, 2003). Therefore, the present study provide a new evidence that alteration of sunlight spectral conditions by colored nets affect differentially the apple fruit growth potential by a combined effect of photosynthetic (leaf photosynthesis) and morphogenetic (leaf area, shoot growth) process on net tree C assimilation rate and carbohydrate availability for fruit growth.

## CONCLUSIONS

Photo-selective nets affected differentially, depending on net color, apple fruit growth. Regardless of PPFD intensity, final fruit size was greater under blue net compared to red net, mainly explained by the positive effect of higher blue light proportion on increases in leaf photosynthesis, leaf area, improving the net tree C assimilation rate, total dry matter production and therefore the availability of carbohydrates for fruit growth. Grey net also improved fruit growth, but the data obtained in this study did not allow explaining with accuracy the physiological aspects involved in this response.

We conclude that shifting the red, blue and far-red light composition by colored nets could be a useful tool to manipulate photosynthetic and morphogenetic process regulating the carbohydrate availability for apple fruit growth.

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**Explorando el uso potencial de mallas foto-selectivas para la regulación del crecimiento de fruto en manzano.** El efecto del sombreado (i.e. reducción de la cantidad de luz solar) sobre la fisiología de crecimiento de fruto ha sido ampliamente estudiado en manzano (*Malus domestica* Borkh.), pero existe poco conocimiento sobre respuestas de crecimiento del fruto a cambios en el espectro de la luz. El objetivo de la presente investigación fue estudiar el efecto del uso de mallas de color con transmisión diferencial de la luz en el espectro azul (B, 400-500 nm), rojo (R, 600-700 nm) y rojo lejano (FR, 700-800 nm) sobre crecimiento de fruto y respuestas fisiológicas asociadas en manzano. Árboles de manzano 'Fuji' de 3 años de edad fueron cubiertos con mallas foto-selectivas azul y roja al 40%, y neutras gris al 40% y blanca al 20% (control). Las mallas azul y roja redujeron en la misma proporción (27%) la radiación fotosintéticamente activa respecto al control. Sin embargo, la malla azul incrementó en 30% y redujo en 10% las relaciones de luz B:R y R:FR, respectivamente. La tasa máxima de crecimiento de fruto bajo las mallas azul y gris fue 15-20% mayor que el control. El peso de fruto bajo la malla azul fue 17% mayor que el control, pero no se encontraron diferencias significativas de peso de fruto entre la malla roja y el control. La fotosíntesis de la hoja y área foliar total bajo la malla azul fueron 28% y 30% más altas que el control, respectivamente; con un consiguiente efecto positivo sobre la asimilación neta de C por el árbol y producción de materia seca total. Los resultados sugieren que cambiar la composición de luz B, R, y FR a través de mallas foto-selectivas podría ser una herramienta útil para manipular los procesos fotosintéticos y morfo-genéticos que regulan la disponibilidad de carbohidratos para el crecimiento de fruto en manzano.

**Palabras clave:** manejo del espectro de la luz solar, tamaño de fruto, fotosíntesis, morfogénesis, *Malus domestica*.

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