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Effect of shading and water stress on light interception, physiology and yield of apple trees

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ABSTRACT

Net shading was explored as a corrective tool for mitigating the effect of a single year drought event on apple ('Imperial Gala') yield in Bologna (Italy). In 2013, trees were grown under three nets with different shading levels (red-50%, white-50%, and black-20%) and without nets. Those treatments received three irrigation doses from 60 days after full bloom until harvest: 260 (control), 115 (moderate water stress, WS) and 50 (severe WS) mm of water. Control trees had midday stem water potential (Ψ_{stem}) values around -1.0 MPa when they were shaded, but values were more negative when they were grown without nets. Ψ_{stem} ranged between -1.0 and -1.5 MPa under moderate WS and was about -1.5 MPa for severe WS although this value was reached sooner in trees grown without nets. Leaf photosynthesis decreased with more negative Ψ_{stem} values but was not affected by shading. Yield was very low (3–4 kg per tree) for trees grown without nets with no differences between irrigation treatments. Yield was also very low for shaded trees grown under severe WS (5–7 kg per tree). Under control and moderate WS, shaded trees had higher yields (9–13 kg per tree) than trees grown without nets, but no differences were found between shading. The benefits of net shading on yield were explained by several additive factors: i) improved water status, ii) delay in fruit maturity giving more time to the fruit to grow, and iii) reduction of photo-inhibition. These results may encourage fruit growers to install nets in their orchards when water is limited. Under severe water stress conditions net shading was not effective and low yields are expected. No effect of net colour was observed but its effect may be observed in a longer term. Further research is necessary to determine the sustainability of shading over multiple years.

1. Introduction

Water scarcity is becoming a major threat to the sustainability of irrigated agriculture in many areas of the world (Feres and Soriano, 2007; Levidow et al., 2014). In years with drought, water shortage can be imposed during a significant period of the irrigation season for numerous crops. In fruit trees, water shortage is extremely counterproductive if it coincides with the end of fruit development (Berman and DeJong, 1996). It is important to implement strategies to compensate the negative effects of water stress on fruit growth in years with drought. Under water stress conditions, fruit growth could be limited by two physiological mechanisms: i) direct limitation of fruit growth because of a reduction in cell turgor in response to water stress (Kramer and Boyer, 1995), and ii) limitation by carbohydrate availability owing to a decrease in photosynthetic rate (Naschitz et al., 2010). A suitable technique for mitigating the adverse effects of water stress on fruit growth should improve tree water status and at least maintain the assimilation capacity of the tree (Lopez et al., 2012).

Net shading has been proposed as a technique to improve tree water status and water use efficiency when water is scarce (Nicolas et al., 2005; Girona et al., 2012; Abouatallah et al., 2012; Esmail et al., 2017; Qi et al., 2017). Although the improvement in plant water status produced a positive effect on leaf photosynthesis in shaded trees grown under water stress conditions (Nicolas et al., 2005), net shading may still have a negative impact on fruit growth capacity because it reduces the amount of light intercepted by the tree (Girona et al., 2012). It is well known that light availability is a primary factor affecting fruit growth (Jackson and Palmer, 1972) and orchard productivity is in general linearly related with the amount of light intercepted by the tree (Giuliani et al., 1998; Rosati and DeJong, 2003). However, all these studies considered the increase of light interception because of the increase of photosynthetically active surface, but limited research has been performed on the effect of modulation of light irradiance on productivity (Raveh et al., 2003; Losciale et al., 2010; Abouatallah et al., 2012; Esmail et al., 2017). Although some research evaluated the combined effects of water stress and net shading on yield in fruit trees (Abouatallah et al., 2012; Esmail et al., 2017), no study has been designed to determine the suitability of net shading as a drought mitigation technique when irrigation availability is lower than the crop water demand.

Fruit growth could depend on multiple interactions between tree water relations, tree light interception, and leaf photosynthesis when trees are shaded under drought conditions. Photo-inhibition could be another important mechanism to consider because fruit growth could benefit from net shading when plants are under saturating light conditions, as it reduces light-induced damages (Blanke, 2009). Under excessive light, photo-protective and recovery processes consume energy and dry matter at the expense of dry matter accumulation (Foyer and Harbison, 1994). Although individual thresholds for fruit growth responses to water stress (Naor, 2012) and light (Rom, 1991; Miller et al., 2015) has been reported, it is still unclear how all these physiological processes interact under different combinations of water stress and net shading conditions and what are the final consequences on marketable yield. There are also gaps of knowledge of how the colour of the net affects such physiological mechanisms, although some studies have already indicated that photo-selective nets modify the anatomy and functionality of the plant and, possibly, fruit growth under well-watered conditions (Shahak et al., 2008; Basile et al., 2012; Bastías et al., 2012).

In this study, shading was explored as drought mitigation technique in apple (*Malus x domestica* Borkh.) because it is the most cultivated temperate fruit tree worldwide (FAOSTAT, 2012) and its fruit growth is very sensitive to water stress (Naor et al., 2008; Girona et al., 2012) with reported moderate limitations in fruit growth when tree water status expressed as midday stem water potential is between -1.0 and -1.5 MPa and strong limitations when these values are lower than -1.5 MPa. Trees were grown under three different shading nets (red and white with 50% of shading and black with 20% of shading) and three

different irrigation treatments. Results were compared with those obtained from trees grown without nets for all the irrigation treatments. Tree water status and light interception as well as leaf gas exchanges and fluorescence were measured to explain possible differences in marketable yield.

2. Material and methods

2.1. Experimental orchard

The experiment was conducted in 2013 in an experimental apple (*Malus x domestica* Borkh. cv. 'Imperial Gala') orchard located at the University of Bologna Experiment Research Station (44°30'N; 10°36'E; 27 m elevation). The orchard consisted of 30 rows with 10 trees per row. The trees (15 years old) were grafted onto 'M9' rootstock and trained to a spindle system. Tree spacing between and within rows was 3.8 and 1.0 m, respectively. The soil volume explored by the root system was calculated as 1.52 m³ from soil profiles from a previous study designed to install soil moisture probes (unpublished results). Root depth was quantified as 0.8 m and root density was quantified as half of the tree spacing (1.9 m²). The orchard had a deep silt-clay soil. Soil water content at field capacity and wilting point was calculated from soil water retention curves of twelve soil samples collected in four different locations in the orchard at four depths (10, 20, 30 and 40 cm) using Richards' pressure plates (Model 1500F1, Soil Moisture Equipment Corp., Santa Barbara, CA) as described by Bitelli (2010). Mean soil water content at field capacity and wilting point was 26% and 12%, respectively. Water holding capacity (amount of water at field capacity) was calculated by multiplying the soil water content at field capacity by the volume of soil explored by the root system (104 mm). The plant available water content was calculated by multiplying the volume of soil explored by the root system by the soil water content at field capacity minus the soil water content at wilting point (56 mm). Trees were irrigated using a drip irrigation system with 2.5 drippers per tree (the distance between emitters was 0.4 m) with a flow of 2.0 L h⁻¹ per dripper. Blooming was observed between the end of March and the beginning of April with full bloom (50% of flowers fully open) observed on April 6. Full canopy development was observed at mid-May. The trees received a commercial management including mineral fertilization to maintain optimal nutrition levels, control of pest and diseases, hand thinning to remove small fruitlets after fruit set, herbicide application below the trees and mowing of inter-canopy cover crop to maintain the desired cover crop height.

2.2. Net shading and irrigation treatments

Nets were installed when the canopy was fully developed to evaluate the interactions between shading and irrigation without interferences with canopy development. On May 17, the orchard was divided in four sectors. One sector (seven rows) remained without nets while the other three sectors were covered with black, red, and white (Polysack Plastic Industries Ltd., Nir Yitzhak, D.N., Negev, Israel) photo-selective hail nets. The black and white nets covered eight rows, while the red net covered seven rows. The nets were placed horizontally over the trees (3.5 m height) to shade all the rows of a given sector. The nets were installed using a supporting tensile structure with longitudinal and transversal steel cables tensioned to a supporting structure of concrete and wood as described by Castellano et al. (2008). The expected relative transmittance of photosynthetically active radiation (PAR) for the black net was 80% while it was 50% for the red and white nets. Each sector received three scenarios of water availability from 60 days after full bloom (DAFB) (June 6) until harvest: control, 45% of

control (moderate WS) and 20% of control (severe WS). These treatments were selected to reproduce different scenarios of water availability for a long period before harvest (about three months) that could be representative of water shortage in years with drought. The irrigation treatments were imposed by modifying the time of irrigation and consequently the amount of water applied. No modification in the flow of the emitters occurred during the season. The three irrigation treatments were randomly assigned to each line for a given sector. Consequently, each sector had a minimum of two lines with the same irrigation treatment (treatment replication). Since the objective of the study was to evaluate the positive effect that nets may have under drought, the amount of irrigation for control trees was calculated to satisfy crop water demand of trees grown under 50% of shading and not for trees grown without nets, assuming a priori that control trees grown without nets may suffer certain water stress under control conditions. To do this, a water balance for replacing crop evapotranspiration ($ET_c = ET_o \times K_c$ effective rainfall) was calculated. ET_o and K_c represent the reference evapotranspiration and crop coefficient, respectively. The Penman-Monteith method was used to determine ET_o (Allen et al., 1998) and K_c values were derived from the empirical relationship between K_c and midday canopy light interception (Ayars et al., 2003). K_c was estimated as 0.6 between June 6 and July 16 and then increased to 0.9 between July 16 and harvest (end of the experiment). Weather data was obtained from the 'Irriframe' network (<http://www.irriframe.it/irriframe>) for the 'Cadriano' weather station located in the University of Bologna Experiment Research Station where the experiment was performed. The weather station was located one km away from the orchard on a grassy surface with no interference of tall obstacles. Effective rainfall was considered the rain after subtracting 5 mm from total rainfall. For each orchard sector, the six central rows were monitored (two rows randomly assigned for each irrigation treatment) leaving one row (trees without nets and red net) or two rows (white and black net) of border trees between sectors to avoid interferences with the adjacent light environments. Within each line (10 trees) the six central trees were monitored and the other trees were considered as border trees. Therefore, a total of 24 experimental rows and 144 trees were monitored (12 trees per each irrigation and sector combination). The volume of water applied to each treatment combination was monitored with twelve volumetric water meters (BETA ALP -SDC 1/2") and it was recorded three times per week during the experiment.

2.3. Tree light interception

Tree PAR interception was measured using a linear ceptometer with an 80 cm-long probe (Accupar Linear PAR, Decagon Devices, Pullman, WA). For each experimental row, two values of incident PAR without interference of the nets, two values of incident PAR below the nets without interference of the trees, and 28 values of PAR below the canopy for a group of three trees located in the central part of the row were taken. The ceptometer was always placed perpendicular to the tree row in a horizontal position. For the 28 measurements performed below the trees, the ceptometer, which was placed just above the soil surface, covered the tree spacing in correspondence with three trees per treatment. Measurements were performed at midday (± 30 min) in completely clear days in four dates (June 6, July 7, July 26 and August 9, corresponding approximately to 60, 90, 110 and 120 DAFB, respectively). For trees grown without nets, PAR intercepted by the canopy was calculated as the difference between mean incident PAR and mean PAR below trees. For trees grown under the nets, PAR intercepted by the canopy was calculated as the difference between mean incident PAR below the nets and mean PAR below trees.

2.4. Midday stem water potential, gas exchanges and chlorophyll fluorescence

Midday stem water potential (Ψ_{stem}) was measured weekly during the experiment using a Scholander-type pressure chamber (Model 3005, Soil Moisture Equipment Corp., Santa Barbara, CA), following the recommendations of [Turner and Long \(1980\)](#). Measurements were taken at solar noon (± 30 min) from leaves located near the crown bases of four experimental trees for each orchard sector and irrigation treatment combination (one leaf per tree). Selected trees were in the middle of central rows for each sector. Each leaf was covered with a plastic bag and aluminium foil for a period of one hour before the measurements. Midday leaf photosynthesis (A_n), leaf conductance (g_s), leaf transpiration (T), intrinsic water use efficiency (WUE) (A_n/T), and chlorophyll fluorescence were measured on May 14 (before net installation), June 7 (after net installation and before the onset of irrigation treatments), on July 9 (mid-way irrigation treatment application), and on July 31 (before harvest). Measurements were taken on the same trees used for Ψ_{stem} determination, but in leaves well exposed to the sun (one leaf per tree) with two LI-6400 portable infra-red gas analyzers (LI-COR 6400, Lincoln, NE, USA) connected to a leaf chamber fluorometer integrated with an LED light source (LI-COR 6400-40, Lincoln, NE, USA). For each net, measurements of gas exchanges were performed by assigning to the LED light source the PAR values detected by the quantum PAR sensor of the LI-6400 below each net. For trees grown without nets, gas exchanges measurements were performed by assigning to the LED light source the PAR values detected in open field conditions. Measurements using natural light were not performed to allow fluorescence and gas exchanges measurements at the same time using the fluorometer leaf chamber. Combining net photosynthesis with chlorophyll fluorescence measurements allowed to perform the quenching analysis as described by [Losciale et al. \(2008, 2011\)](#) determining three traits: i) J_{PSII} , the electron transport rate exiting from PSII, ii) J_{CO_2} , the electron transport rate “fixed” by means of net photosynthesis, and iii) J_{NC} , the residual electron transport rate exiting from PSII and funnelled to non-net carboxylative processes like photorespiration and alternative electron transports.

2.5. Total and marketable yield

Harvest was performed when fruit achieved their commercial maturity to avoid possible interactions between fruit maturity and growth. Maturity was monitored using a Da-meter (Model DA-53500, Turoni, Forlì, Italy). Two times before harvest, 20 fruits per each orchard sector and irrigation combination were sampled from several trees to determine their maturity index. Fruit were harvested when the mean output of the DA-meter had a value lower than 0.9, indicative of mature ‘Gala’ apple ([Costamagna et al., 2013](#)). A total of 12 trees for each sector and irrigation combination were harvested. For each tree, crop load (total number of fruit per tree) and total and marketable yield (Kg tree^{-1}) were determined. Marketable yield was calculated taking into consideration fruit with a diameter higher than 65 mm. Fruit diameter of each harvested fruit was measured and recorded using calipers connected to an external memory (Calibit; www.calibit.it/).

2.6. Statistical analysis

The effect of shading, irrigation and their interaction on Ψ_{stem} , A_n , g_s , T , WUE, chlorophyll fluorescence traits, and marketable yield was evaluated by a two-way analysis of variance (ANOVA). HSD’s test was used to separate the mean values that were significantly different for all the shading and irrigation treatment combinations to facilitate the interpretation of possible interactions between irrigation and shading using a one-way ANOVA. The effect of crop load and mean Ψ_{stem}

during the experiment on total and marketable yield was explored by analysis of regression. Relationships between Ψ_{stem} and A_N were also explored by regression analysis. The version 3.0.0 of the R software (R Development Core Team, 2012) was used for the statistical analysis. Tree light interception was not subjected to statistical analysis because a single value was calculated for each irrigation and shading treatment combination (value derived from 28 measurements for three trees).

3. Results

3.1. Rainfall and applied water

Accumulated effective rainfall between early April and June 6 was 145 mm and trees received no irrigation (Fig. 1). During this period accumulated ET_0 was 220 mm. From June 6 until August 20 (harvest time) accumulated ET_0 was 398 mm and 30 mm of effective rainfall were registered (Fig. 1). During this period, control trees received 260 mm of water trough irrigation. Moderate WS trees received 115 mm and severe WS trees 50 mm. The total amount of effective rainfall during the experiment was 175 mm while the total rainfall observed was 330 mm due to 31 rain events registered between April and August 20. The effective rainfall estimated in this study represented a 53% of total rainfall.

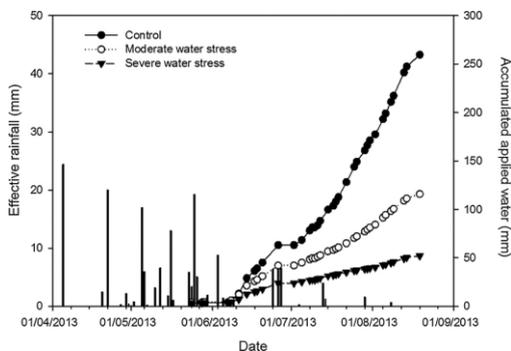


Fig. 1. Seasonal patterns of applied water for ‘Imperial Gala’ apple grown under three irrigation treatments. Vertical bars indicate effective rainfall.

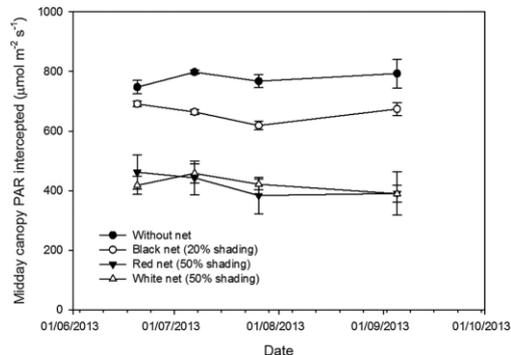


Fig. 2. Seasonal patterns of photosynthetically active radiation (PAR) intercepted by the canopy for ‘Imperial Gala’ apple grown without nets and shading nets. Each point represents the mean canopy PAR interception for a group of three trees for the three irrigation treatments.

3.2. Tree light interception

Midday incident PAR was 1980, 2015, 1860, and 1635 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on June 6, July 7, July 26, and August 9, respectively. Trees grown without nets were exposed to those PAR values and had the highest values of midday PAR intercepted by the canopy ($\sim 800 \mu\text{mol m}^{-2} \text{s}^{-1}$), followed by trees grown under the black net ($\sim 680 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Fig. 2). Trees grown under the red and white nets had the lowest values of PAR intercepted by the canopy ($\sim 400 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Fig. 2).

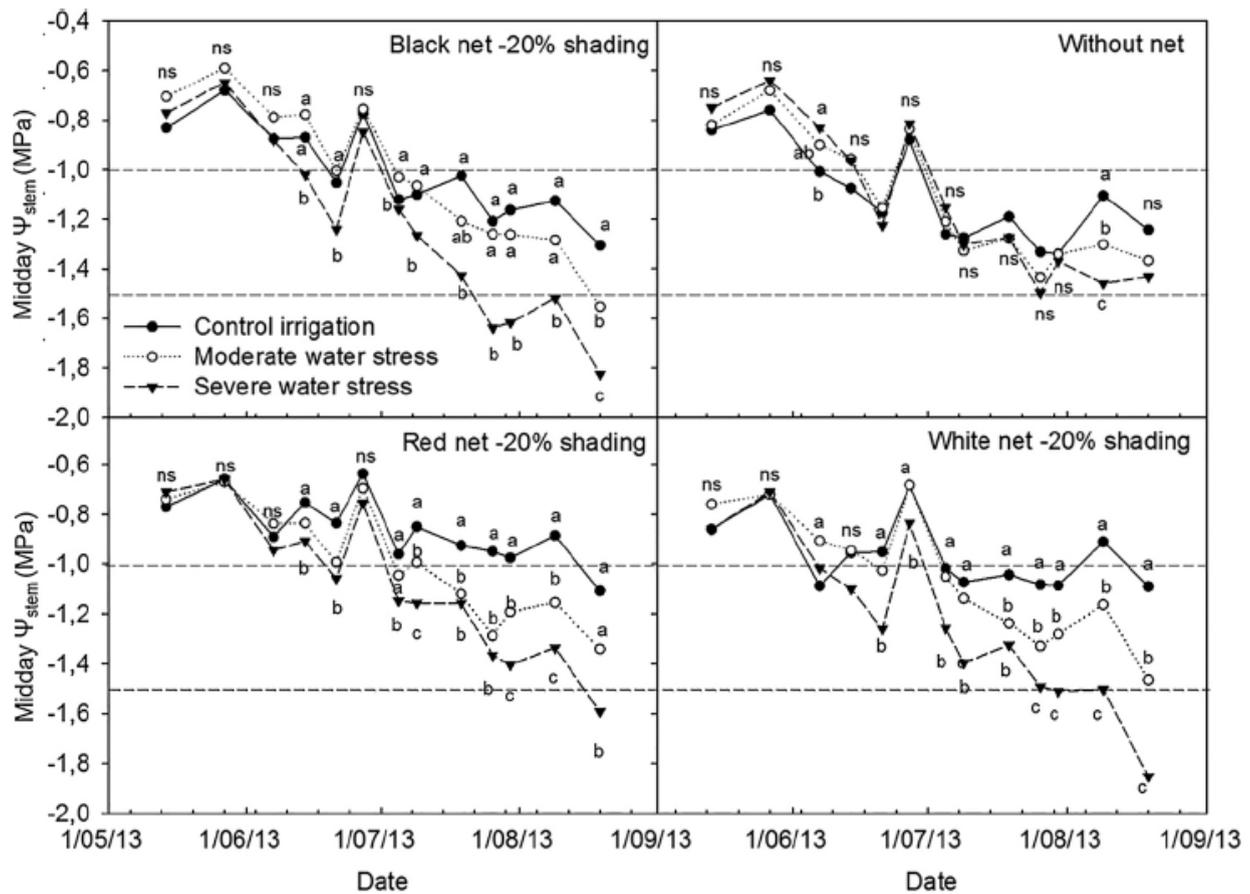


Fig. 3. Effect of irrigation treatments on midday stem water potential for ‘Imperial Gala’ apple grown without nets and shading nets. Each point represents the mean value of four trees. For each day, treatment’s means followed by different letters are significantly different at 5% according to HSD’s test. ns indicate not significant differences between treatment’s means. Dashed line indicates theoretical thresholds for apple growth responses to water stress according to Naor et al., (2012) (> -1.0 MPa: no limitation, between -1.0 and -1.5 MPa moderate limitation, and < -1.5 MPa strong limitation).

3.3. Seasonal patterns of midday stem water potential

All trees had similar midday Ψ_{stem} before the application of irrigation treatments (June 6) (Fig. 3). After June 6, slight differences in Ψ_{stem} were observed between irrigation and shading treatments, but differences disappeared after several rain events (30 mm of accumulated effective rainfall) (Figs. 1 and 3). Between June 26 and August 20 (harvest), shading and

irrigation influenced Ψ_{stem} (Fig. 3). Regarding the general effect of irrigation, no differences in Ψ_{stem} between irrigation treatments were observed for trees grown without nets, except on August 9 when severe WS trees had lower values than control trees (Fig. 3). Severe WS trees had lower Ψ_{stem} than control trees when they were grown under shading nets, while moderate WS trees had intermediate Ψ_{stem} values (Fig. 3). Regarding the effect of shading for control trees, similar patterns of Ψ_{stem} (~ -1.0 MPa) were observed under the three types of nets, except for the days immediately prior to harvest, when trees grown under the black net reduced their values (Fig. 4). Trees grown without nets had the lowest values of Ψ_{stem} in comparison with the three nets (minimum values of -1.3 MPa) (Fig. 4). Regarding the effect of shading under moderate WS, Ψ_{stem} values ranged between -1.0 and -1.5 MPa for trees grown under the three types of nets but trees grown without nets had the lowest values of Ψ_{stem} (Fig. 4). Regarding the effect of shading under severe WS, Ψ_{stem} values for all the trees (trees grown under the three nets and without nets) were around -1.5 MPa for almost one month before harvest without differences between shading (Fig. 4).

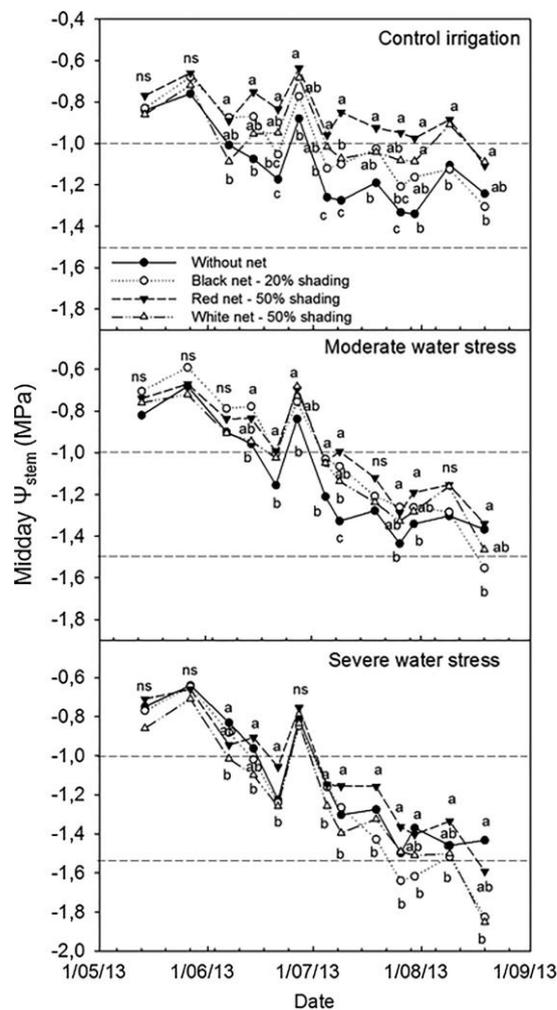


Fig. 4. Effect of shading on midday stem water potential for ‘Imperial Gala’ apple grown under three irrigation treatments. Each point represents the mean value of four trees. For each day, treatment’s means followed by different letters are significantly different at 5% according to HSD’s test. ns indicate not significant differences between treatment’s means. Dashed line indicates theoretical thresholds for apple growth responses to water stress according to Naor et al., (2012) (> -1.0 MPa: no limitation, between -1.0 and -1.5 MPa moderate limitation, and < -1.5 MPa strong limitation).

3.4. Gas exchanges and chlorophyll fluorescence

Before net installation (May 14) no differences in A_n , g_s , T and any chlorophyll fluorescence traits were observed between trees (results not shown). When the measurements were performed on June 7, July 9 and July 31, incident PAR in the LICOR-6400 leaf chamber was set to 1560, 1780 and 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. The respective values for trees grown under the black net (20% shading) were 1365, 1380 and 1398 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and for the white and red net (50% shading) 875, 996, and 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Those values represented the incident PAR above the tree on those specific dates.

Between net installation and the onset of irrigation treatments (June 7), A_n , g_s , and T values were very low with no significant differences between trees (Tables 1 and 2). No significant differences were observed for WUE either (Table 2). One month after the application of the irrigation treatments (July 9) A_n , g_s , and T increased in comparison with the previous date (Tables 1 and 2). Control trees grown under the white net had higher A_n values than severe WS trees under the black net but no differences were observed among the other treatments (Table 1). No significant differences were also observed for g_s , T and WUE (Table 2). On July 30, a high variability in gas exchanges values was observed between trees (Tables 1 and 2). A linear relationship was found between Ψ_{stem} and A_n on July 30 (Fig. 5). This produced a reduction in A_n values of trees grown without nets due to their negative Ψ_{stem} (Table 1). The lowest values were observed for control trees grown under severe WS (Table 1). Within each irrigation treatment, there were no differences in A_n between trees grown under 20% of shading (black net), and 50% of shading (white and red net) (Table 1). A similar trend was observed for g_s and T , while WUE did not show significant differences between trees (Table 2).

The amount of electrons funnelled to net photosynthesis (J_{CO_2}) followed the same patterns of A_n presented in Table 1 as it has the same meaning but expressed as electron transport rate. Their values ($\mu\text{mol m}^{-2} \text{s}^{-1}$) ranged between 14.3 and 26.2 on June 7, 34.9 and 61.1 on July 9, and 38.1 and 70.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on July 30. Regarding J_{PSII} no differences were observed between trees on June 7 and July 9 (Table 1).

On July 31, when the effect of irrigation treatments on Ψ_{stem} was evident, J_{PSII} was similar among the three nets under control and moderate WS conditions (Table 1). But under severe WS, control trees without net had the lowest J_{PSII} values (Table 1). For J_{NC} no differences were observed between trees during the whole experiment (Table 1).

3.5. Total and marketable yield

Measurements of fruit maturity determined two harvest dates: August 13 for trees grown without nets and August 20 for trees grown under the nets. There was a high variability in crop load from tree to tree (between 50 and 250 fruits per tree) (Fig. 6A,C), perhaps explained by the rainy conditions during bloom time that might have prevented a homogenous insect pollination. Total yield increased with crop load (Fig. 6A) and marketable yield was reduced with more negative values of Ψ_{stem} (Fig. 6D). There was a significant interaction between shading and irrigation for marketable yield ($P < 0.05$). Trees grown without nets had the lowest marketable yield for all the irrigation treatments (Fig. 7). Trees grown under the three nets had similar marketable yield with one exception under control conditions: lower values for trees grown under the white net in comparison with trees grown under the black net (Fig. 7).

Treatments	June 7 (Onset irrigation)			July 9 (Midway application irrigation)			30 July (Two weeks before harvest)		
	A _n	J _{PSII}	J _{NC}	A _n	J _{PSII}	J _{NC}	A _n	J _{PSII}	J _{NC}
Irrigation	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	*	n.s.
Shading	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	n.s.
Irrigation x shading	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Control									
Without net	3.5 a	88.9 a	74.6 a	11.2 ab	133.2 a	88.24 a	13.8 abc	149.5 ab	94.3 a
Black-20%	4.4 a	85.0 a	67.2 a	13.0 ab	178.6 a	126.5 a	16.5 ab	157.9 a	91.3 a
Red-50%	6.0 a	83.7 a	59.6 a	14.6 ab	161.4 a	102.9 a	16.0 ab	141.5 ab	77.4 a
White-50%	6.3 a	101.3 a	76.0 a	15.2 a	166.5 a	105.4 a	17.5 a	165.8 a	95.6 a
Moderate water stress									
Without net	4.2 a	84.1 a	67.4 a	12.6 ab	159.4 a	109.3 a	12.3 abc	141.7 ab	92.3 a
Black-20%	4.0 a	74.2 a	58.5 a	13.7 ab	165.2 a	110.1 a	15.7 ab	163.7 a	100.3 a
Red-50%	4.4 a	73.7 a	56.2 a	12.7 ab	160.0 a	109.3 a	14.4 abc	156.6 a	99.4 a
White-50%	5.9 a	101.5 a	77.8 a	14.2 ab	171.4 a	114.4 a	12.6 abc	152.8 ab	102.3 a
Severe water stress									
Without net	4.7 a	88.4 a	69.1 a	11.6 ab	154.1 a	107.8 a	9.5 c	112.5 b	74.4 a
Black-20%	4.3 a	81.5 a	64.5 a	8.7 b	157.0 a	122.1 a	12.6 abc	156.8 a	106.5 a
Red-50%	4.5 a	81.4 a	63.4 a	10.3 ab	152.2 a	110.8 a	11.1 bc	125.4 ab	81.1 a
White-50%	6.5 a	99.7 a	73.6 a	11.4 ab	165.5 a	119.8 a	12.2 abc	153.9 ab	105.0 a

Table 1

Effect of irrigation and shading on leaf photosynthesis (A_n), the electron transport rate exiting from PSII (J_{PSII}), the residual electron transport rate exiting from PSII and funneled to non-net carboxylative processes like photorespiration and alternative electron transports (J_{NC}) at three different times during the experiment (June 7, July 9, and July 30). All traits are expressed in $\mu\text{mol m}^{-2}\text{s}^{-1}$. For any column, means followed by different letter and asterisk (*) indicates significant differences at 5% according to HSD's test. n.s. = no significant.

Treatments	June 7 (Onset irrigation)			July 9 (Midway application irrigation)			July 30 (Two weeks before harvest)		
	g _s	T	WUE	g _s	T	WUE	g _s	T	WUE
Irrigation	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	n.s.
Shading	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	n.s.
Irrigation x shading	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Control									
Without net	29.5 a	0.918 a	3.922 a	186.9 a	5.032 a	2.325 a	215.4 ab	5.675 ab	2.469 a
Black-20%	35.4 a	1.041 a	4.146 a	197.1 a	4.908 a	2.648 a	228.7 ab	5.566 abc	3.042 a
Red-50%	69.4 a	1.816 a	3.443 a	223.1 a	5.408 a	2.709 a	250.0 ab	5.808 ab	2.711 a
White-50%	54.3 a	1.481 a	4.285 a	234.9 a	5.574 a	2.742 a	293.7 a	6.922 a	2.545 a
Moderate water stress									
Without net	36.8 a	1.091 a	3.510 a	169.6 a	4.628 a	2.702 a	147.2 ab	4.269 bcd	2.893 a
Black-20%	34.4 a	0.991 a	3.734 a	188.6 a	4.708 a	2.923 a	207.6 ab	5.171 abcd	3.046 a
Red-50%	38.5 a	1.052 a	4.106 a	185.3 a	4.616 a	2.791 a	196.5 ab	4.825 bcd	3.027 a
White-50%	49.3 a	1.351 a	4.407 a	199.6 a	4.954 a	2.876 a	166.8 ab	4.597 bcd	2.789 a
Severe water stress									
Without net	43.9 a	1.317 a	3.766 a	125.5 a	3.876 a	2.990 a	126.6 b	3.562 cd	2.694 a
Black-20%	40.3 a	1.150 a	3.734 a	116.9 a	3.282 a	2.749 a	148.6 ab	3.887 bcd	3.307 a
Red-50%	38.3 a	1.061 a	4.247 a	152.4 a	4.057 a	2.557 a	129.5 b	3.413 d	3.205 a
White-50%	55.4 a	1.498 a	4.309 a	160.3 a	4.351 a	2.717 a	139.9 b	3.867 bcd	3.166 a

Table 2

Effect of irrigation and shading on leaf conductance (g_s) ($\text{mmol m}^{-2}\text{s}^{-1}$), transpiration (T) (mmol) and intrinsic water use efficiency (WUE) (A_n/T, $\mu\text{mol mmol}^{-1}$) at three different times during the experiment (June 7, July 9, and July 30). For any column, means followed by different letter and asterisk (*) indicates significant differences at 5% according to HSD's test. n.s. = no significant.

4. Discussion

Marketable yield, that in apple generally considers only fruit size, is considered the most interesting trait for the fruit industry, so it was selected as the target parameter to evaluate tree productivity in response to shading and irrigation in this study. The rest of the physiological (tree light interception, Ψ_{stem} , A_n, g_s, T and chlorophyll fluorescence) and agronomic traits (crop

load and harvest date) were monitored to explain potential differences in marketable yield in response to shading and water stress.

Marketable yield was negatively affected by WS (Figs. 6 and 7) because apple fruit growth is very sensitive to any reduction in irrigation that imposes midday Ψ_{stem} lower than -1.0 MPa for a long period before harvest (Naor, 2012). The reduction in fruit growth in this study may be explained in part by a limitation in A_n (Table 1, Fig. 6) due to stomatal closure (Table 2) in response to WS (Naschitz et al., 2010). In addition, the decrease of Ψ_{stem} in response to WS (Fig. 3 and 4) could have impacted fruit growth by reducing xylem sap flows to growing fruit (Drazeta et al., 2001). Marketable yield was extremely low under severe WS (between 3 and 4 kg tree) (Figs. 6 and 7) when trees experienced Ψ_{stem} of about -1.5 MPa for a long period before harvest, as previously reported by Naor (2012) (Fig. 4). Low marketable yields were also observed under control irrigation and moderate WS for trees grown without nets (Fig. 7), indicating that apple production without nets is not economically feasible for the levels of irrigation applied in the experiment (Fig. 1) that were much lower than the E_{T0} (398 mm). This result is in good agreement with other studies that reported reductions in yield when applied irrigation was lower than tree requirements (Ebel et al., 2001; Girona et al., 2010). However, using net shading allowed a significantly higher production of fruit under control and moderate WS in comparison with trees grown without nets (Fig. 7). The results of this study may indicate the suitability of reducing the light interception of the trees when shading is able to maintain midday Ψ_{stem} between -1.0 and -1.3 MPa for a long period before harvest in spite of water shortage. However, when water availability is very low (50 mm applied for the severe WS treatment, Fig. 1) net shading was not enough alone to relieve water stress (Fig. 4) and mitigate the negative effect of WS on marketable yield (Fig. 7). Under severe WS it may be necessary to combine shading with other mitigation techniques such as leaf removal or severe fruit thinning (Lopez et al., 2012).

Net shading was applied once the tree canopy was fully developed to establish links between shading, water stress and fruit growth without any interaction with tree vegetative development. Under this experimental condition, the benefits of net shading on fruit growth may be explained by several additive factors: i) improved water status, ii) delay in fruit maturity, and iii) reduction of photo-inhibition. Perhaps the main factor was the improved water status for trees grown under the nets in comparison with trees grown without nets when a certain amount of water was still available in the orchard (control and moderate WS in Figs. 3 and 4). Another benefit could be related to the delay in harvest of one week in trees grown under the nets and consequently their fruit had an extra week to accumulate water and carbohydrates. Fruit from trees grown under nets would have been exposed to lower temperatures as previously reported in other studies (Basile et al., 2012). This could have reduced fruit growth rates in comparison with trees without nets and delayed the ripening process. Under the conditions of this experiment, with midday incident PAR values close to $1800\text{--}2000 \mu\text{mol m}^{-2} \text{s}^{-1}$, which are much higher than the $1000\text{--}1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ threshold for leaf A_n saturation reported in apple (Jackson, 2003), another benefit of net shading could be the reduction of photo-inhibition with consequent lower losses of carbohydrates for photo-protection and recovery, due to a reduction of excessive light. In general, even if trees grown without net intercepted significantly more light than trees grown under the nets (Fig. 2), the electron transport rate exiting from PSII (JPSII) in their leaves was similar than in those of the shading treatments (Table 1), suggesting that leaves from trees grown without nets were not able to use the extra energy they received for photochemistry as well as for photosynthesis. Probably the excessive light not used in the electron transport chain was dissipated via the non-photochemical quenching processes (Losciale et al., 2011). On July 9, A_n decreased with severe WS while JPSII remained quite similar and consequently the activity of non net carboxylative processes like photo-respiration and the alternative electron transports (JNC) increased (Table 1). On July 30, when the different water treatments

were fully differentiated, trees subjected to severe WS grown without nets had a decrease of both A_n and JPSII, while JNC remained stable (Table 1). The behaviour observed on July 9 and 31 suggested the effective role of these photo-protective mechanisms under excessive light and WS (Flexas and Medrano, 2002) never reported in apple before.

Another important issue to take into account when installing a net to mitigate the negative effect of drought in an orchard is the percentage of shading. The effects of shading in several processes such as vegetative growth, leaf photosynthesis, flower fruit initiation, fruit quality and yield have been studied in apple to determine how much light is necessary for maximum orchard performance. It has been stated that light interception for fruit production and tree management is optimised at 70 to 80% of available light (Rom, 1991; Miller et al., 2015). However, the maximum level of shading that an apple orchard can tolerate under drought is not well known. For this reason, in this experiment two levels of shading were applied (20% with a black net and 50% with a red and white net). Although increasing shading from 20% to 50% (Fig. 2) was high enough to improve tree water status and marketable yield under control and moderate WS, in comparison with trees grown without nets (Figs. 3,4 and 7), there was a similar tree performance in marketable yield under 20 and 50% of shading. This may indicate that it is possible to reduce solar radiation between 20% and 50% in years with drought in apple producing areas with high irradiance such as Bologna. It would be interesting in future experiments to increase the shading levels of this experiment (higher than 50%) to study light thresholds for apple physiological traits and fruit size under water stress conditions. It would be also interesting to test the results under different environmental conditions and on years with very high incoming radiation, as the year when this study was performed was very cloudy and rainy (31 days of rain during the experiment) (Fig. 1).

Regarding net colour, from the results of this study, we can not derive any clear conclusion about the effect of the colour in the physiological and agronomical performance of the trees. All the traits evaluated in this study were similar between the red and the white net with the same percentage of shading. One of the most important effects of colour has been previously reported in the canopy development and the morphology of leaves (Bastías et al., 2012). However, in our study the nets were installed when the canopy was almost fully developed to avoid interactions between shading and plant morphology during the first part of the growing season. Further research over multiple years may be required to determine if net colour plays a significant role on apple production and to determine the sustainability of shading in fruit tree orchards, including of bloom return, fruit set, canopy size and fruit quality information. For this one-year study it can be concluded that shading can be proposed as a corrective solution to mitigate the negative effect of water stress in apple orchards.

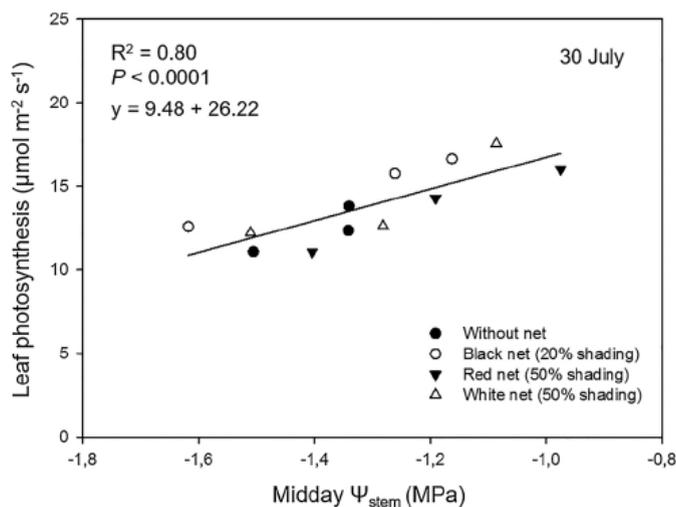


Fig. 5. Relationship between midday stem water potential and leaf photosynthesis for ‘Imperial Gala’ apple before harvest (30 July). Each point represents the mean value of four trees. The relationship was fitted to a linear regression.

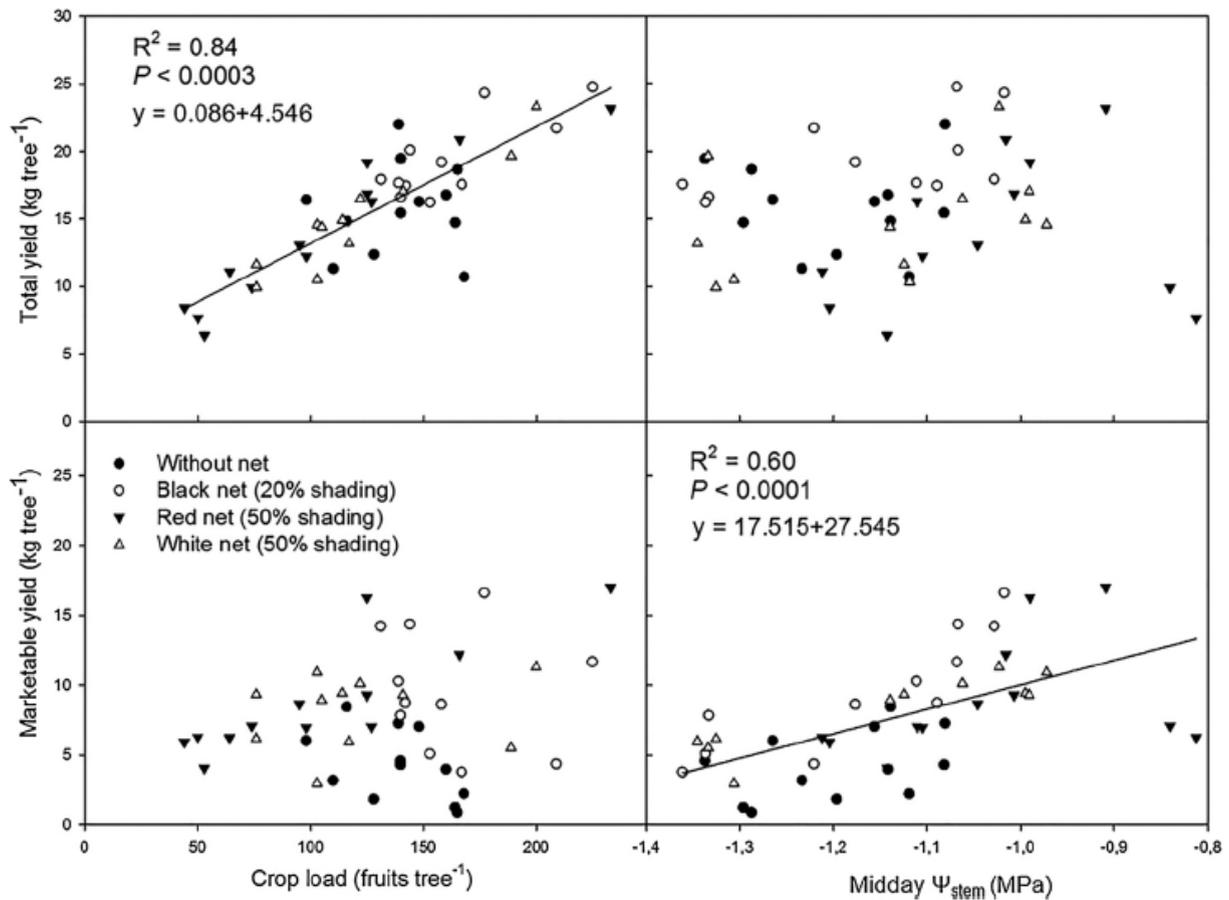


Fig. 6. Relationships between crop load and total yield (A) and marketable yield (C) and between mean midday stem water potential and total yield (B) and marketable yield (D) for ‘Imperial Gala’ apple. Each point represents one tree. Significant relationships were fitted to a linear regression.

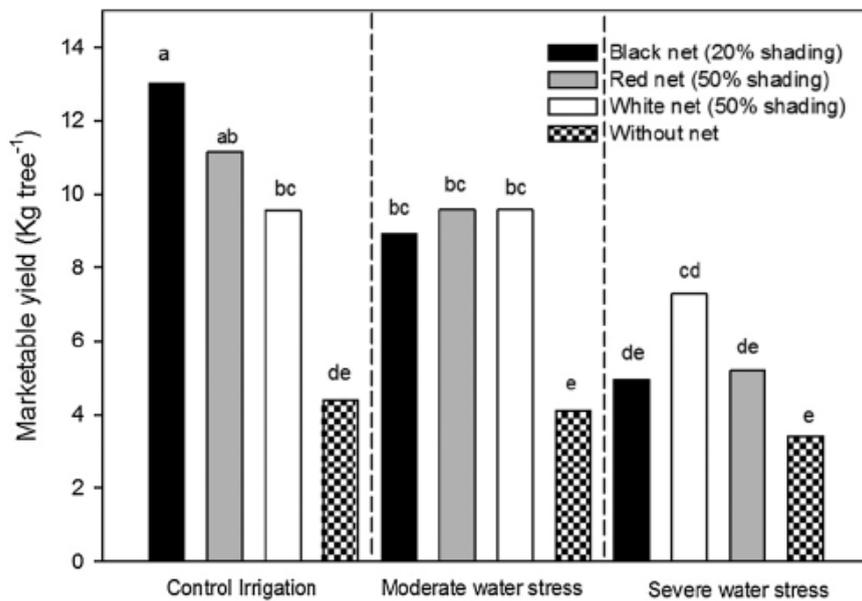


Fig. 7. Effect shading on marketable yield for ‘Imperial Gala’ apple grown under three irrigation treatments. Each bar represents the mean value of 12 trees. Means followed by different letters are significantly different at 5% according to HSD’s test.

5. Conclusions

When trees received lower irrigation requirements than the expected crop water needs, trees grown under shading nets had a better water status than trees grown without nets. Leaf photosynthetic activity was not reduced by shading, which appears to be due to the beneficial effect of reducing excessive photon pressure on the leaves. The benefit in tree water status along with the maintenance of leaf photosynthesis allowed to have higher marketable yields for trees grown under the nets than in trees grown without nets. Increasing shading from 20% to 50% only slightly benefited tree water status while it did not affect leaf photosynthesis and marketable yield. This may indicate that if apple trees are exposed to an excess of light, such as in Mediterranean regions, they can still perform well until a light reduction of 50%. Shading delayed the date of harvest by one week in comparison with trees grown without nets giving an extra week to fruit grown under the nets to accumulate water and carbohydrates. Fruit growers should take this delay into consideration depending on their production objectives, as precocity is generally associated with higher prices in apple. No effect of net colour in any leaf physiological trait was expected in this study considering that the nets were installed when tree canopy was fully developed. Further research over multiple years may also be necessary to determine the optimum colour of the nets, but we can conclude from this study that shading can be proposed as a corrective technique to mitigate the negative effects of water stress on fruit growth due to three additive factors: i) improved water status, ii) delay in fruit maturity giving more time to the fruit to grow, and iii) reduction of photo-inhibition. However, if the levels of water stress are severe for a long period before harvest (about -1.5 MPa or lower expressed as midday water potential), net shading may not be effective for mitigating the negative effect of water stress and low yields could be expected. Although this study focused on the mitigation of water stress under drought conditions it seems apparent that net shading can be extremely useful to reduce irrigation needs and save water.

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