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1 Early apple fruit development under photosensitive nets.

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7 Abstract

8 The objective of this 2-year study (2017, 2019) was to evaluate the influence of photosensitive
9 nets on apple fruit growth, focusing on the initial fruit growth stages of “*Pink Lady*”. Trees were
10 subjected to four photosensitive nets (Blue, Red, White and Yellow) and a standard black one
11 (serving as Control), resulting in 5 light environments (LE), all shading at 20%. From 20 to 90
12 DAFB, 32 fruit and extension shoots, for each LE, were measured for a total of 11 times during
13 the season. For each LE, fruit gauges were also installed to monitor fruit daily growth
14 parameters, from 50 to 90 DAFB. At harvest, all fruit from each light environment were
15 weighed and quality parameters were measured on 40 fruit per treatment. For each year,
16 correlations were made to test the influence of LEs on final fruit weight and quality, and the
17 impact that extension shoot growth had on fruit growth. In both years, white and control nets
18 led to the production of fruits with higher weight compared to the other treatments (blue, red
19 and yellow nets). In 2019, LEs did not appear to influence the relationship between shoot and
20 fruit growth, probably due to the higher crop load and the massive pruning carried out the
21 previous year. The higher vegetative outburst in this year might have unbalanced resources
22 towards the shoots, than towards the fruit. Results show how weather and orchard management
23 heavily influenced the trees responses. Yet, the consistent findings of final fruit weight, for both
24 years, indicate that different wavelengths influence fruit and shoot behaviour, even at early
25 phenological stages. Hence, growers searching for bigger fruit should refer to white and black

26 nets. Further studies approaching this technology can help improve apple production
27 management and knowledge of the use of photoselective nets.

28
29 KEYWORDS: apple, orchard, photoselective nets, fruit growth, fruit gauges, shoot growth,
30 yield.

31 32 1. INTRODUCTION

33 To overcome the adverse effects of climate change, the use of protective nets, also called
34 anti-hail nets or shade nets, are becoming a common practice, since they protect orchards from
35 excessive solar radiation and hail damage. Such net installations are advisable in regions with
36 a high incidence of extreme weather events such as hail, often occurring in the Mediterranean
37 basin (Iglesias and Alegre, 2006; Özkaya et al., 2018).

38 Since protective nettings reduce solar radiation, they also modify the orchards'
39 microenvironment (Green et al. 2003). Netting also protects leaves from excessive light leading
40 to higher photosynthetic efficiency (Murata et al. 2006; Lopez et al. 2018). The most commonly
41 used types of net are black and white, whose physiological responses are widely known. The
42 addition of chromatic particles in the nets thread modifies light quality, thus its spectra,
43 direction, diffusion, reflectance, transmittance and absorbance (Basile et al. 2008; Ganelevin,
44 2008; Shahak, 2008), plus its scattering, that can increase by 17-170% depending on the net
45 (Abdel-Ghany and Al-Helal, 2010). The use of photoselective nets has been studied in many
46 horticultural species (Stamps, 2009; Basile et al. 2012; Zoratti et al. 2015; Aoun and Manja,
47 2020; Gullo et al., 2021), but researchers are focusing on the impact of photoselective netting
48 in apple production systems.

These nets have proven to modify the orchard microenvironment with effects on relative humidity, air and soil temperature (Iglesias and Alegre, 2006; Solomakhin and Blanke, 2010; Arthurs et al. 2013; Kalcsits et al. 2017). They also directly affect sap flow rates, regardless of the shading power (Boini et al. 2019). The orchard's geographic latitude location is, nevertheless, the main variable that influences on the microenvironmental changes.

In apple, during the very early stages of fruit development, fruitlets are known to possess active stomata (Blanke and Lenz, 1985), which contribute to active fruit transpiration. However, in first half of the season, fruit growth is directly related to a high xylem functionality. This will cause water backflows from fruit to stem when leaf transpiration losses are very high, followed by strong rehydrations during late afternoon and night, when stomal conductance is reduced (Lang, 1990; Morandi et al. 2012). In the second part of the season, fruit growth is almost completely sustained by phloem flow, while the xylem vessels lose their functionality. This marks the transition from an exponential growth model, to a linear one (Lakso et al. 1995). The first, at the beginning of the season, is mainly characterized by cell division and an initial stage of increasing growth rates. This phenological phase is known to highly influence final fruit dimensions, as cell number can be a primary factor for fruit size (Lakso et al. 1989). The second phase is characterized by cell expansion, therefore constant growth rates will last until harvest, only with slight deaccelerations during the central parts of the day (Lang, 1990).

In light of the above information, it can be said that photoselective netting can be applied to apple orchards, to improve certain aspects of its physiology. However, there appear to be no studies focusing on the early stages of fruit growth. Considering the potential influence of these nets on many physiological traits, assumptions can be made that there may be an impact on fruitlet development and growth. Thus, the first objective of this study was to investigate on the

74 responses of fruit growth during cell division stage, under different photoselective nets, on a
75 daily and seasonal scale, to understand if light spectrum can modify early stages of fruit growth,
76 with consequences on final yield.

77 An important factor to keep into consideration during early fruit growth is the marked presence
78 of shoot elongation rates. Vegetative growth is known to negatively influence fruit
79 development, being a stronger sink, generally competing for photosynthetates (Grappadelli et
80 al. 1994). Given this unbalance in such a delicate phase that dictates final production, the second
81 objective of this study was to: understand if coloured nets can modify the competitive
82 relationship between vegetative and reproductive growth.

83 Results would allow to highlight or identify the possibility to optimize cell division stage, in
84 order to improve fruit quality and yield, in apple modern production. Insights of apple crop
85 responses to different light environments would also contribute to the pool of information
86 related to apple physiology under the effect of different light spectra.

88 2. MATERIALS AND METHODS

89 *2.1. Field and experimental set-up.* The trial took place at the experimental farm of the
90 University of Bologna, located in Cadriano (Bologna) (44°30'N; 10°36'E; 27 m elevation), in
91 an experimental apple orchard (*Rosy Glow*) grafted on M9, in seasons 2017 and 2019. Trees
92 were planted in 2015, spaced 3.3x1.0 m, with 10 rows of 50 trees each. Trees were trained as
93 the solaxe system in 2017, then modified to an open canopy system, between 2018 and 2019.
94 The orchard rows were North-South oriented, with concrete poles 3.8 meters high, sustaining
95 iron wires at three heights. Full bloom dates were 30 March 2017 and 29 March 2019. Around
96 20 days after full bloom (DAFB), in both years, four photoselective nets (red (R), blue (B),
97 white (W) and yellow (Y)) (ChromatiNet®, Polysack Industries, Negev, Israel) were placed

over sections of individual rows, as a mono-row cover, above 4 trees each (plus 2 serving as guard trees). All four photosensitive nets mesh measured 0.4x0.2 cm, had an English-turn weaving system and had a shading percentage of around 20% (Corollaro, 2014). To avoid interference between treatments, areas of around 11 meters in length and width were left completely uncovered, around each plot (manufacturer's suggestion). The rest of the orchard was covered by a classic black anti-hail net (C) (Valente, Campodarsego, PD, Italy), as a roof system, which served as control; this net measured 2.8x8 cm, had an English-turn weaving system and shaded 20% (manufacturer statement). Also C had 4 monitored trees and these were selected taking into account 11 meters distance from the coloured nets, plus another 11 meter distance from the beginning of the forenamed net cover. The choice of such nets installation comes from a limited amount of available space in the orchard (1500 m²). The generated five light environments (LEs) were repeated twice, thus, the trial was set to create a complete randomized block design, where each LE had 8 monitored trees. The trial set up lasted until harvest, in 2017; after harvest, coloured nets were taken off from the rows, while the control net was rolled up, on top of the rows, a common practice in commercial orchards for Italy. In 2019, the trial set up lasted until the end of June (90 DAFB), after which, the whole orchard was covered with the classic black hail net, thus all trees received the same quantity and quality of light. It is assumed that LEs did not affect flower bud differentiation between seasons 2017 and 2019, since during year 2018 the orchard did not undergo any sort of trial (all trees were covered with the same black control net).

2.2. Light spectra assessment. In winter 2017, a LI1800 spectroradiometer (LI-COR, Lincoln, NE, USA) was used to assess the irradiance of the five LEs, on a cloudless day, at midday and outside the orchard. The wavelength interval was between 400 and 1100 nm and portions of each net were placed 20 cm over the sensor. Wm⁻² outputs were converted to μmol

$\text{m}^{-2} \text{s}^{-1}$; transmittance was then calculated as the ratio between the external light input and the light intensity under the net and expressed as a percentage.

2.3. Weather parameters. Meteorological conditions were measured using a weather station (Wi-Net s.r.l. Cesena, Italy) installed outside the orchard. The following weather parameters were recorded every 15 minutes: temperature, relative humidity, solar radiation, wind speed and rain. Weather data was used to calculate environmental vapor pressure deficit (VPD) and solar radiation (W m^{-2}) was converted to photosynthetically active radiation (PAR) ($\mu\text{mol m}^{-2} \text{s}^{-1}$).

2.4. Fruit growth. From around 20 to 90 DAFB, in both years, fruit growth was monitored both with a caliper and with automatic fruit gauges, for continuous measurements.

2.4.1. Caliper measurements. For each LE, 32 random fruit (4 tree^{-1}) were tagged and their diameter was recorded with a digital caliper, connected to an external memory (www.hkconsulting.it). Measurements were taken twice a week until the end of May, then once a week in June, for a total of 11 times. At each measurement date, diameter data (D) from all monitored fruit were converted to fresh weight (FW) using the following conversion equation:

$$\text{FW(g)} = a * \text{D(mm)}^b \quad (1)$$

where a and b were $0.0006 (\pm \text{SE } 0.00005)$ and $2.924 (\pm \text{SE } 0.0194)$. This equation was obtained by regressing diameter and weight data of about 300 fruit picked from various *Pink Lady* apple orchard (R^2 of the relationship was >0.99). Fruit absolute growth rate (AGR), expressed as g day^{-1} , was then calculated using the following equation:

$$\text{AGR}_{t1} = (\text{FW}_{t1} - \text{FW}_{t0}) / (t_1 - t_0) \quad (2)$$

2.4.2. Continuous fruit growth measurements. For each LE, 8 fruit were randomly selected and their diameter was continuously measured using custom-built fruit gauges, as described by

Morandi et al. (2007). These consisted of a variable linear resistance transducer, supported by a stainless-steel frame (Megatron Elektronik AG & Co., Munchen, Germany). A wireless data logger system (Wi-Net s.r.l. Cesena, Italy) (Giorgetti et al. 2014), composed of five nodes, received data from the fruit-gauges and communicated with a central coordinator. The network coordinator acted as a gateway towards the internet, through a general packet radio service (GPRS) modem.

Fruit were monitored at 15-minute intervals. At each monitoring interval, diameter data (D) from all monitored fruit were converted to FW using the conversion equation (1). Thereafter, fruit AGR was then calculated using the equation (2), thus by accumulating the hourly AGR, fruit daily growth was then obtained, expressed in g day^{-1} .

In season 2017, technical issues did not allow to obtain data for the first 20 days after the installation and made it impossible to collect data throughout the season from the B LE. For homogeneity reasons, in both years, data are shown from approximately 50 to 90 DAFB. For this continuous period of 40 days, the following fruit growth parameters were calculated for each LE: hourly mean AGR (AGR_{hour}), mean gained weight, daily gained weight, mean maximum AGR (AGR_{max}) and mean minimum AGR (AGR_{min}).

During these 40 days, four periods were selected: 50, 60, 70 and 90 DAFB. For each period, up to 3 consecutive days were used as replicates (when meteorological conditions were the same), to create daily patterns of fruit growth, in terms of AGR. For each period, the five previously mentioned fruit growth parameters were further tested for each LE.

In general, when fruit gauges showed excessive noise or error values, they were excluded from the data set.

2.5. Extension shoot measurements. From 20 to 90 DAFB of both years, 32 extension shoots were randomly selected for each LE (4 tree⁻¹) and their extension was traced twice a

week until the end of May, then once a week during June. Measurements on shoots were collected on the same dates as those for fruit' with the caliper. At each measurement date, length data (L) from all monitored shoots allowed to calculate the absolute extension rate (AER), in terms of mm day^{-1} , adapting equation (2) by substituting FW with L.

2.6. Harvest and fruit quality. At harvest (around 205 DAFB in 2017 and 207 DAFB in 2019) all fruit from each LE were calipered. Thereafter, the application of equation (1) allowed to estimate the average fruit weight for each LE. Fruit quality parameters were measured on 40 representative fruit LE^{-1} (20 replication⁻¹). A slice of each fruit was used for soluble solid content (SSC, °Brix), by measuring the refractometric index of the juice, with a HI 96811 digital refractometer (Hanna, Woonsocket, RI), and for relative dry matter (RDM, %), calculated as the percentage of dry weight relative to fresh weight, after drying samples in a forced air oven at 60°C.

2.7. Correlations study. For each year, the following correlations were obtained.

- i) Average fruit weight and quality parameters (SSC and RDM) were related to fruit growth parameters obtained from fruit gauges, during the continuous 40-day period.
- ii) Fruit growth parameters obtained from fruit gauges, during the continuous 40-day period, were related to crop load.
- iii) Fruit AGR for each LE, obtained with caliper measurements, were related to the extension shoot AER.

2.8. Statistical analysis.

2.8.1. Light spectra assessment. A one-way ANOVA was used for separating the averages among blue light band (400–500 nm), green (500–600 nm), yellow-red band (600–800 nm), PAR (400–700 nm) and infrared band (IR) (> 700 nm).

2.8.2. *Weather*. A one-way ANOVA was applied for evaluating differences every 10 days for environmental VPD and mean PAR between 9 and 18 h, between the two seasons.

2.8.3. *Continuous fruit growth measurements*. A one-way ANOVA was used for testing differences between LEs for fruit growth parameters, for each year. A two-way ANOVA was used for testing differences between LEs fruit AGR_{hour}, during daily time, for each year.

2.8.4 *Extension shoot measurements*. A one-way ANOVA was used for testing differences among LEs.

2.8.5. *Harvest parameters*. Crop load was analyzed between treatments, revealing no significant difference for each year, thus a one-way ANOVA could be used to test differences for average fruit weight.

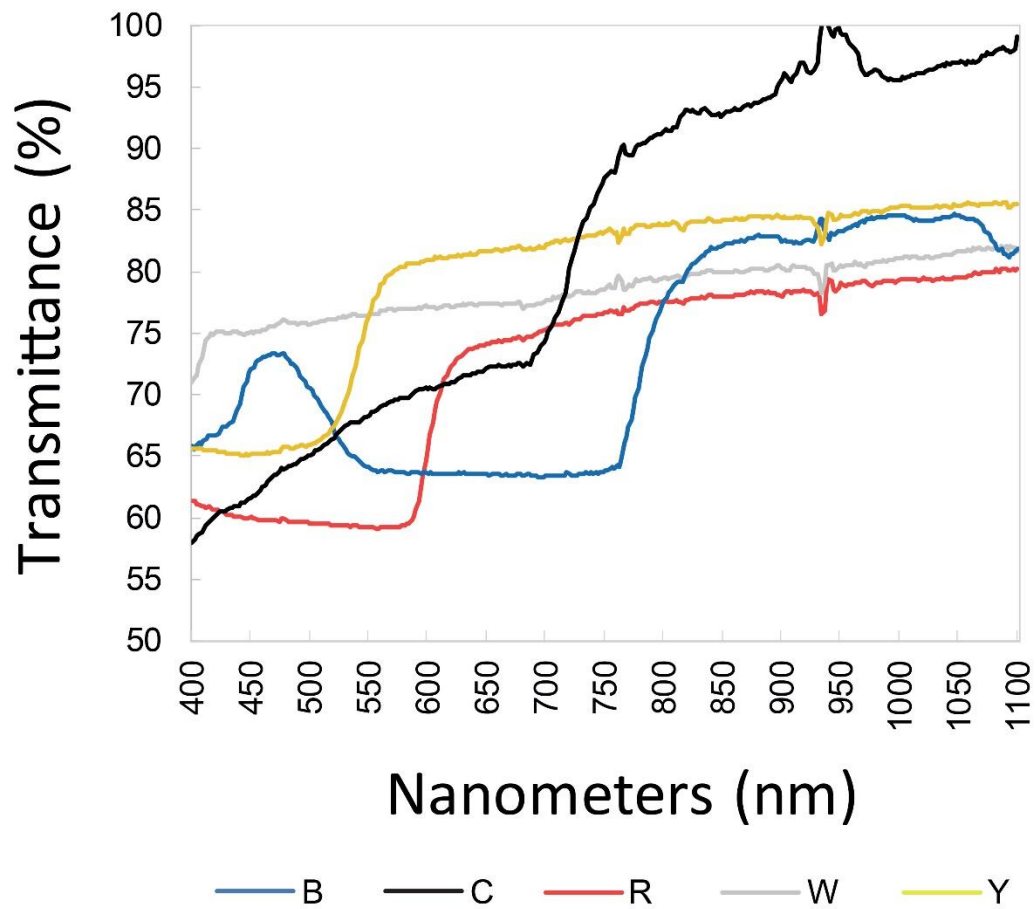
2.8.6. *Correlations study*. Correlations i) and ii) were analyzed and the correlation coefficients, slopes and p-values reported. Regarding correlation iii), each LE relation was analyzed, reporting the correlation coefficients and p-values. A repeated measures analysis followed, after which slopes were tested for the effects of LE, AER, time and AER*time.

SNK's test (Zar, 1984) was then applied to rank the averages, in all the reported analyses, considering significant $P < 0.05$.

3. RESULTS

3.1. *Light spectra assessment*. Spectral distribution of light transmitted under tested nets (Fig. 1) showed transmittance patterns typical of their different colour. In the blue (400-500 nm) (Fig. 2A) and green (500-600) areas (Fig. 2B) every treatment was different, W having highest transmission and R the lowest, leaving the other LEs alternating as intermediates. In the yellow-red area (600-800) (Fig. 2C) Y was highest and B lowest, while in the infra-red region

216 (< 700 nm) (Fig. 2D) values were higher for C and lower for B and R. PAR (400-700 nm) (Fig.
217 2E) was more transmitted under the W net (around 75%) and less under the R LE (around 65%).



218

219 *Figure 1. Spectral light transmittance under nets, ranging from 400-1100 nm.*

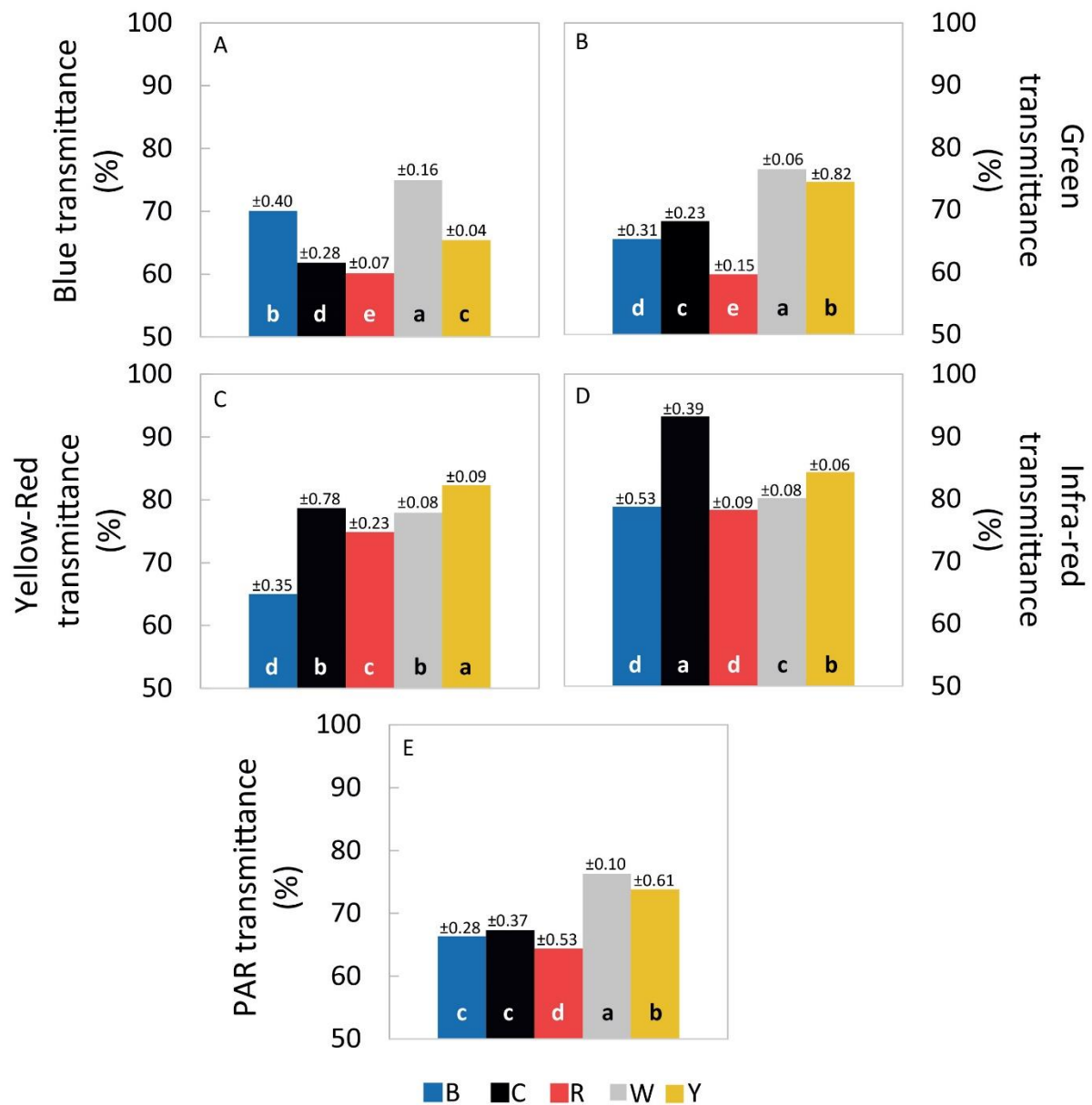


Figure 2. Transmittance mean values for blue (A), green (B), yellow-red (C), infra-red (D) and PAR (E) wavebands, under each LE. Different letters indicate significant difference at $P < 0.05$. Numbers on top of each column are standard error values.

3.2. Weather parameters. Between the two years, 2017 showed generally higher PAR and VPD values, especially from 40 DAFB on.

In 2017, from 23 to 61 DAFB, the maximum solar radiation was stable around $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$, moreover, it reached peaks of over $2300 \mu\text{mol m}^{-2} \text{s}^{-1}$, while the average PAR, measured

229 between 9 and 18 h, was about $1150 \mu\text{mol m}^{-2} \text{s}^{-1}$, but exceeded $1300 \mu\text{mol m}^{-2} \text{s}^{-1}$, 11 times
230 during the season. The period going from 62 to 91 DAFB was characterized by slightly lower
231 peaks of maximum solar intensity (peaks above $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ only 10 times) and the mean
232 PAR was about $1280 \mu\text{mol m}^{-2} \text{s}^{-1}$, reaching peaks above $1300 \mu\text{mol m}^{-2} \text{s}^{-1}$ 22 times (Fig. 3A)
233 during the season.

234 In 2019, the weather conditions were considerably different. From 20 to 63 DAFB, the solar
235 radiation was frequently below $800 \mu\text{mol m}^{-2} \text{s}^{-1}$, reaching peaks over $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ just 4
236 times and a single one of over $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$. The second period, from 64 to 91 DAFB was
237 characterized by higher solar intensity peaks (peaks above $1300 \mu\text{mol m}^{-2} \text{s}^{-1}$ almost every day)
238 and the average PAR was about $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 3A).

239 Also, VPD was different between the two years, especially from 40 DAFB to 60 DAFB. In
240 2017 the average of this period was 0.75 kPa, while in 2019 the medium was 0.35 kPa. The
241 average recorded value in 2017, from 20 to 90 DAFB, was 0.88 kPa and in the same period of
242 2019, VPD reaches the same value (0.87 kPa) (Fig. 3B).

243

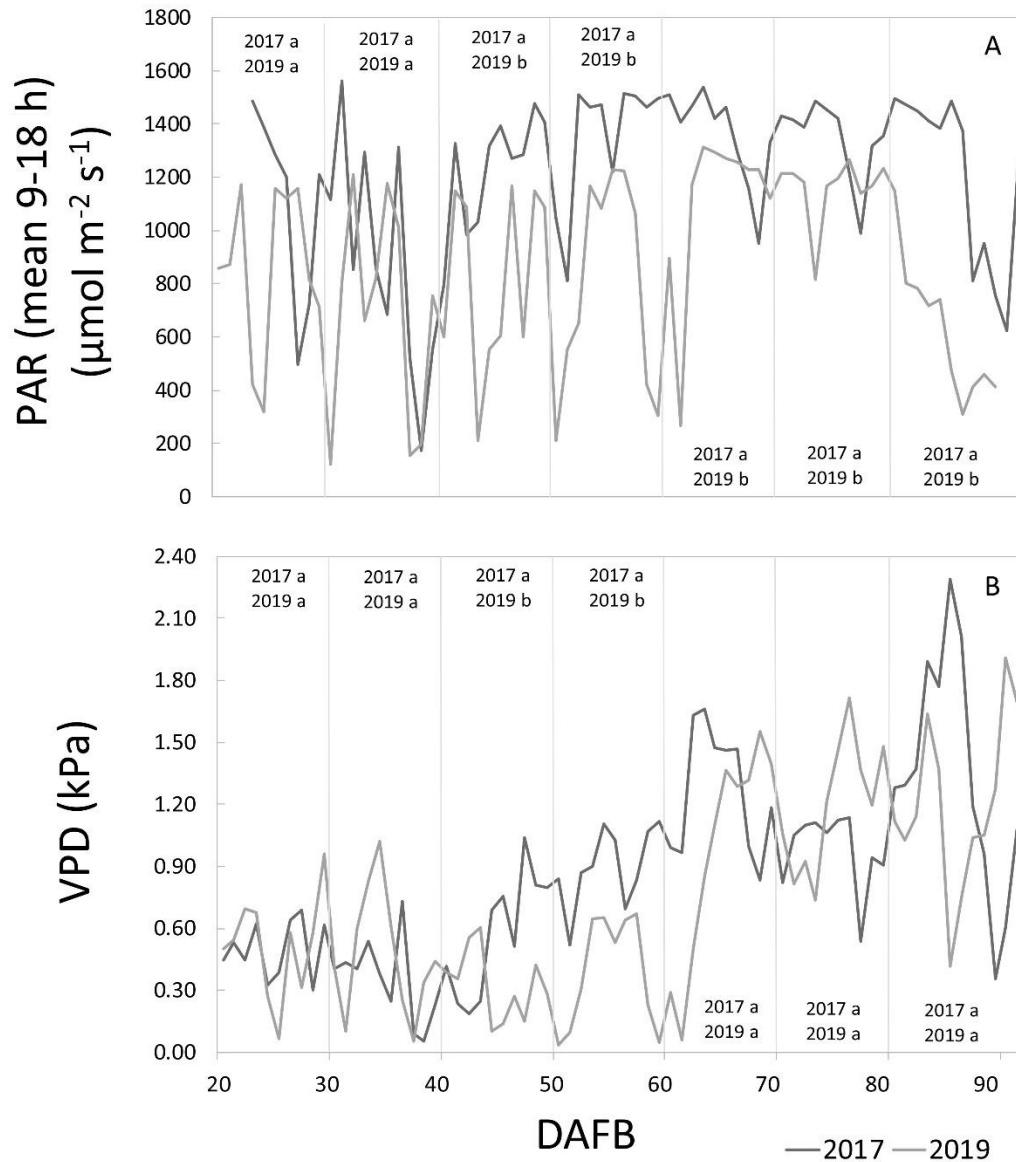


Figure 3. Comparison of mean PAR between 9-18h, for 2017 and 2019 referred to DAFB (A). Comparison of VPD in the two years, referred to the DAFB (B). The presence of different letters indicates significant difference at $P < 0.05$.

3.3. Fruit growth. Daily fruit growth (Fig. 4) showed significant differences. In 2017, at 50 DAFB, daily growth and most fruit growth parameters were similar for each LE (Fig. 4A, Table 1), with periods of rapid growth recorded in the late afternoon and at night, followed by periods of reduced or even negative growth during the central part of the day (Fig.4A). Only Y had the highest mean gained weight (0.187 g day^{-1}). At 61 DAFB, there was a significant

253 difference between LEs, more precisely, Y tended to have higher values (Fig. 4C, Table 1),
254 possibly because of a higher AGR_{max} (Table 1).

255 With fruit development, shrinkage was replaced by a steady state for some LEs (at 77 DAFB
256 and 91 DAFB) (Fig. 4E-G). At 77 DAFB, significant differences can again be found, and the R
257 treatment presents a significant rehydration, from 17:00 to about 24:00 h and the mean gained
258 weight was significantly higher (0.63 g day^{-1}). On this date, R and W had a midday shrinkage
259 phase, compared to C and Y where there was no shrinkage, however with no differences during
260 most of the day. Yet, Y net also reached high mean gained weight values(0.69 g day^{-1}), like R,
261 a possible result given by the lack of shrinkage. At 91 DAFB the situation was similar among
262 all treatments, although W growth exceeded 1.20 g day^{-1} (Table 1), unlike B (Table 1) which
263 had lower values for AGR_{hour} and fruit daily growth.

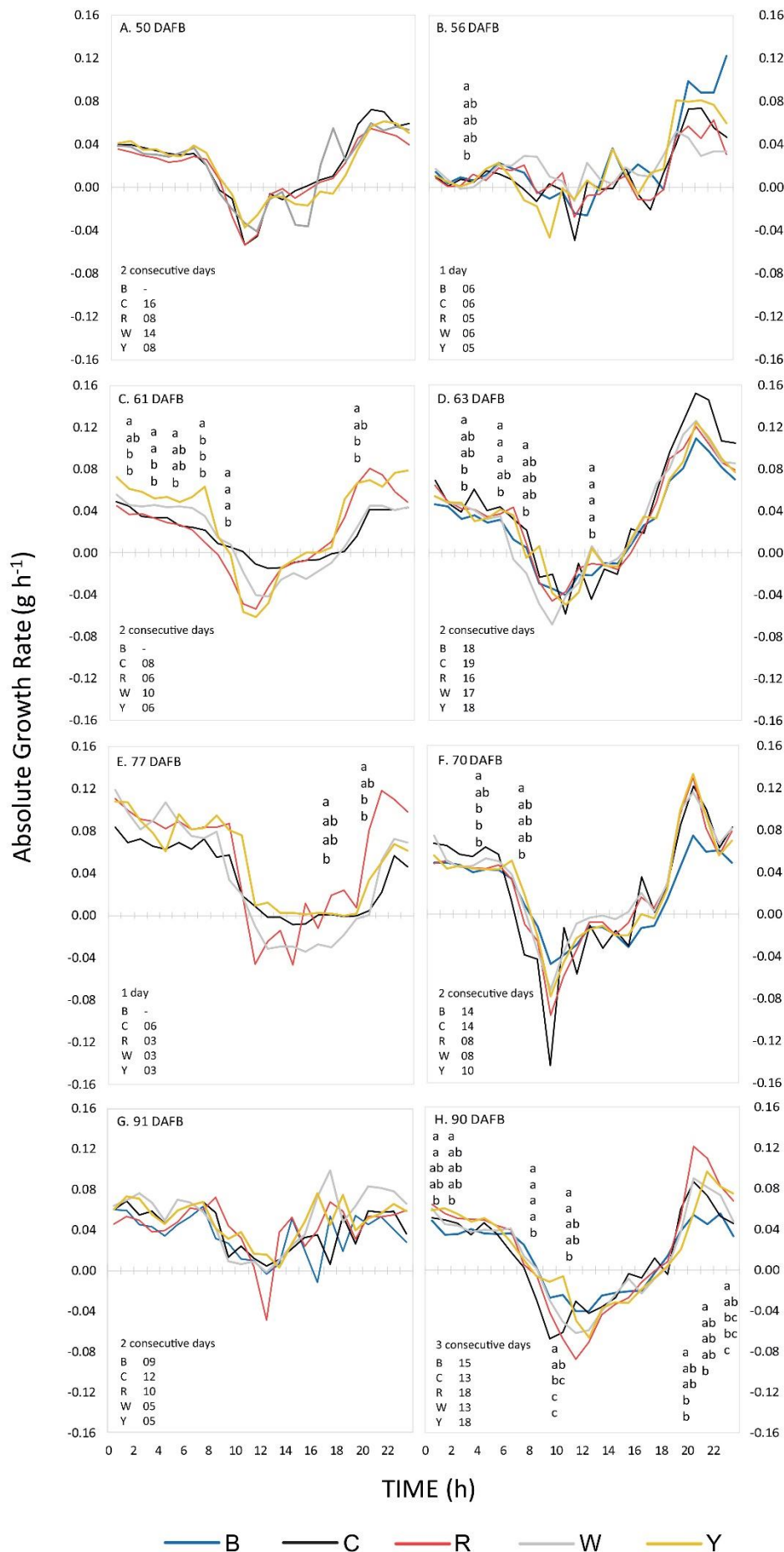


Figure 4.

Fruit daily AGR, for season 2017 (on the left) and season 2019 (on the right), under B (blue lines), C (black lines), R (red lines), W (light grey lines) and Y (yellow lines) LEs, at four significant periods.

For each period, the number of days used for analyses and the number of used fruit gauges, for each LE, are reported in the bottom left corner.

Different letters indicate significant difference at $P < 0.05$. No letters indicate no significant differences.

298 In 2019, at 56 DAFB (Fig. 4B) the trend was not characterized by marked contractions in the
 299 central hours of the day, however growth rates were very low, without differences between LEs.
 300 Nevertheless, table 1 shows higher fruit mean gained weigh under the W (0.150 g day⁻¹) net
 301 and lower ones under the C (0.044 g day⁻¹).
 302 A week later (Fig. 4D), daily growth increased, showing peaks of rehydration during the night
 303 and early morning. During these hours, there were significant differences among LEs, whereas
 304 in the middle of the day all treatments seemed to react in the same way. W tended to have the
 305 lowest values from 3:00 to 12:00 h. Fruit growth parameters at 63 DAFB (Fig. 4D, Table 1),
 306 were significantly different, C having higher values especially when compared to B and W.
 307 At 70 DAFB (Fig. 4F), midday shrinkage was still present, with no marked differences among
 308 LEs. Yet, C presents the lowest value, in terms of mean gained weight (0.128 g day⁻¹) (Table
 309 1), nearly half compared to W (0.224 g day⁻¹).
 310 In the last period, at 90 DAFB (Fig. 4H), in the first hours of the day, Y fruit grew more than
 311 the other LEs, then, before midday C and R tended to shrink more, along with W (values below
 312 -0.05 g hour⁻¹) ones. B fruit had intermediate behaviour firstly, whilst became the lowest by the
 313 end of the day, in terms of g hour⁻¹. Among fruit parameters, differences were present for mean
 314 gained weight, where Y was highest (0.165 g day⁻¹) and C was lowest (0.07 g day⁻¹).

315
 316 *Table 1. Average fruit growth parameters (horizontally listed) values, followed by SE, for each light environment*
 317 *(vertically listed) in 2017 and 2019. For each date, different letters among rows indicate significant difference at*
 318 *P<0.05. ns indicates no significance.*

		AGR _{hour}				Mean gained weight			Total gained weight			AGR _{max}			AGR _{min}		
Year	DAFB	LE	(g h ⁻¹)	SE		(g day ⁻¹)	SE		(g day ⁻¹)	SE		(g)	SE		(g)	SE	
2017	50	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		C	0.021	0.007	ns	0.165	0.022	ab	0.447	0.044	ns	0.076	0.006	ns	-0.067	0.013	ns
		R	0.015	0.006	ns	0.135	0.017	c	0.350	0.014	ns	0.056	0.004	ns	-0.060	0.017	ns
		W	0.018	0.006	ns	0.152	0.019	bc	0.395	0.056	ns	0.078	0.007	ns	-0.072	0.013	ns
		Y	0.018	0.006	ns	0.187	0.019	a	0.412	0.035	ns	0.067	0.005	ns	-0.058	0.010	ns

2019	61	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		C	0.017	0.004	b	0.189	0.016	b	0.356	0.081	ns	0.067	0.012	b	-0.027	0.007	ns	
		R	0.018	0.008	b	0.120	0.022	c	0.378	0.061	ns	0.088	0.007	ab	-0.059	0.008	ns	
		W	0.016	0.007	b	0.203	0.018	b	0.335	0.111	ns	0.089	0.014	ab	-0.073	0.015	ns	
		Y	0.029	0.009	a	0.293	0.030	a	0.629	0.055	ns	0.119	0.009	a	-0.075	0.026	ns	
	77	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		C	0.034	0.007	ns	0.489	0.042	b	0.731	0.151	ns	0.099	0.018	ns	-0.008	0.004	ns	
		R	0.052	0.011	ns	0.630	0.057	a	1.133	0.186	ns	0.147	0.012	ns	-0.073	0.032	ns	
		W	0.035	0.011	ns	0.483	0.037	b	0.659	0.328	ns	0.130	0.032	ns	-0.039	0.026	ns	
		Y	0.050	0.008	ns	0.693	0.063	a	1.093	0.120	ns	0.122	0.008	ns	-0.003	0.002	ns	
	91	B	0.035	0.004	b	0.428	0.048	b	0.840	0.178	ns	0.129	0.028	ns	-0.057	0.047	ns	
		C	0.041	0.004	ab	0.510	0.054	ab	0.979	0.162	ns	0.120	0.025	ns	-0.038	0.019	ns	
		R	0.043	0.005	ab	0.488	0.058	ab	0.990	0.143	ns	0.135	0.021	ns	-0.091	0.046	ns	
		W	0.052	0.006	a	0.582	0.070	a	1.275	0.121	ns	0.146	0.026	ns	0.011	0.020	ns	
		Y	0.050	0.004	ab	0.569	0.063	ab	1.108	0.219	ns	0.126	0.013	ns	-0.005	0.013	ns	
	2019	56	B	0.023	0.008	ns	0.110	0.028	ab	0.542	0.141	ns	0.093	0.012	ns	-0.039	0.005	ns
			C	0.012	0.006	ns	0.044	0.016	c	0.284	0.095	ns	0.090	0.017	ns	-0.081	0.029	ns
			R	0.012	0.005	ns	0.071	0.014	bc	0.274	0.058	ns	0.102	0.013	ns	-0.059	0.013	ns
			W	0.018	0.003	ns	0.150	0.025	a	0.413	0.168	ns	0.096	0.028	ns	-0.043	0.017	ns
			Y	0.018	0.007	ns	0.069	0.025	bc	0.425	0.071	ns	0.105	0.021	ns	-0.060	0.031	ns
63		B	0.027	0.009	b	0.167	0.033	c	0.601	0.080	b	0.160	0.014	ns	-0.069	0.013	ns	
		C	0.041	0.012	a	0.286	0.053	a	0.983	0.109	a	0.213	0.029	ns	-0.100	0.016	ns	
		R	0.034	0.010	ab	0.243	0.042	b	0.785	0.058	ab	0.161	0.012	ns	-0.081	0.013	ns	
		W	0.031	0.011	b	0.194	0.042	c	0.741	0.055	ab	0.165	0.016	ns	-0.102	0.011	ns	
		Y	0.033	0.009	ab	0.237	0.039	b	0.747	0.050	ab	0.163	0.015	ns	-0.083	0.014	ns	
70		B	0.016	0.007	ns	0.149	0.019	bc	0.338	0.136	ns	0.107	0.014	ns	-0.093	0.017	ns	
		C	0.021	0.012	ns	0.128	0.031	c	0.446	0.116	ns	0.185	0.034	ns	-0.149	0.021	ns	
		R	0.022	0.010	ns	0.146	0.029	bc	0.489	0.101	ns	0.163	0.021	ns	-0.113	0.033	ns	
		W	0.030	0.009	ns	0.224	0.032	a	0.638	0.074	ns	0.162	0.019	ns	-0.088	0.025	ns	
		Y	0.024	0.010	ns	0.195	0.023	ab	0.450	0.076	ns	0.119	0.011	ns	-0.091	0.015	ns	
90		B	0.013	0.007	ns	0.126	0.017	b	0.259	0.046	ns	0.084	0.005	b	-0.061	0.007	a	
		C	0.013	0.009	ns	0.077	0.023	c	0.248	0.071	ns	0.134	0.008	a	-0.149	0.016	c	
		R	0.017	0.012	ns	0.107	0.030	bc	0.342	0.042	ns	0.142	0.011	a	-0.107	0.009	b	
		W	0.014	0.009	ns	0.110	0.023	bc	0.287	0.051	ns	0.107	0.011	ab	-0.076	0.006	ab	
		Y	0.018	0.009	ns	0.165	0.022	a	0.363	0.049	ns	0.115	0.010	ab	-0.082	0.010	ab	

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3.4. Extension shoot measurement. The average behaviour of shoots during the

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monitored periods was different between the two years. In 2017, extension shoot growth did

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not show significant differences (Table 2) and all the values were between 0.21 mm day⁻¹ (B)

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and 0.28 mm day⁻¹ (R).

In 2019, there were significant differences between the LEs (Table 2). The highest values were obtained under B (0.86 mm day⁻¹) and R (0.88 mm day⁻¹), different from W (0.65 mm day⁻¹) and even more different from C (0.54 mm day⁻¹) and Y (0.50 mm day⁻¹) (Table 2).

Table 2. Values of shoot extension growth (mm day⁻¹) for 2017 and 2019, followed by SE. Different letters indicate significant difference at $P < 0.05$, while ns indicates no significance.

LE	25-91 DAFB (2017)			26-90 DAFB (2019)		
	mm day ⁻¹	SE		mm day ⁻¹	SE	
B	0.20	0.019	ns	0.87	0.038	a
C	0.25	0.021	ns	0.54	0.036	c
R	0.26	0.019	ns	0.88	0.040	a
W	0.25	0.021	ns	0.64	0.035	b
Y	0.25	0.020	ns	0.51	0.033	c

3.5. Harvest, fruit weight and quality.

In 2017, the total load did not present differences and all the values were between 50 and 80 fruit tree⁻¹ (Fig. 5A). Fruit weight values ranged from 182 to 202 g and all LEs were significantly different from each other (Fig. 5B). The highest value was reached with W (202.37 g), the lowest with B (182.75 g), while the other LEs were close to 190 g.

B, R and Y fruit showed the lowest SSC, with values around 13.5 °Brix, while C and W, reached 14.5 and 14 °Brix (Fig. 6A), respectively. Instead, RDM did not present significant differences with all values above or close to 17% (Fig. 6B).

In 2019, the situation changed. The total load went from 130 to 180 fruit tree⁻¹ (Fig 5C), and this affected the total marketable yield, in fact, the highest average fruit weight value was 163.73 g, for fruit grown under C net, while the lowest reached around 150 g, under both R and Y nets (Fig. 5D).

Regarding SSC and RDM, the first were significantly higher in B and C with values close to 12.5 °Brix, while the other treatment resulted lower, less than 12 °Brix (Figure 6C). RDM (Fig.

6D) showed significant differences among LEs: B showed higher values (0.25%) than C, W and Y which were similar, while all treatments were higher than R (0.23 %).

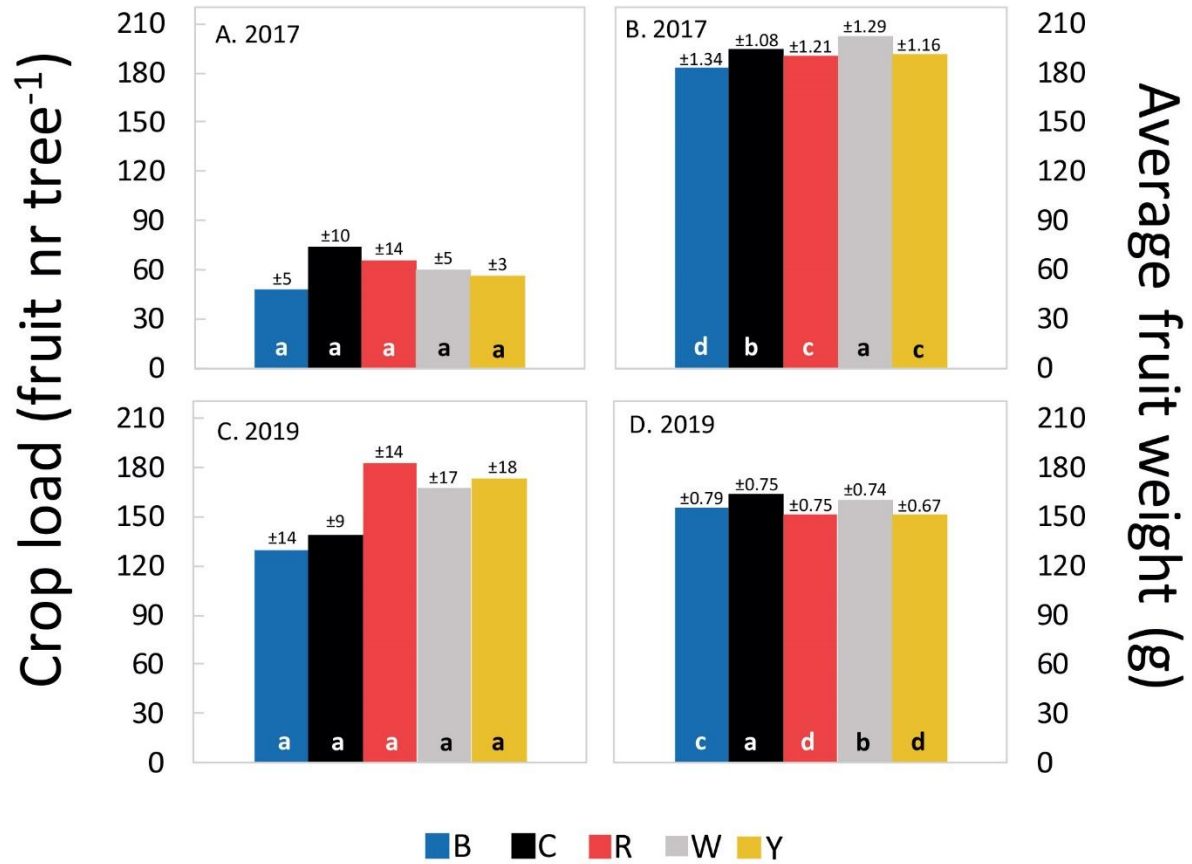


Figure 5. Crop load and average fruit weight of blue (B), control (C), red (R), white (W) and yellow (Y) LEs. Plots A and B are referred to 2017, while C and D are referred to 2019. Different letters, among columns, indicate significant difference at $P < 0.05$. Numbers on top of each column are standard error values.

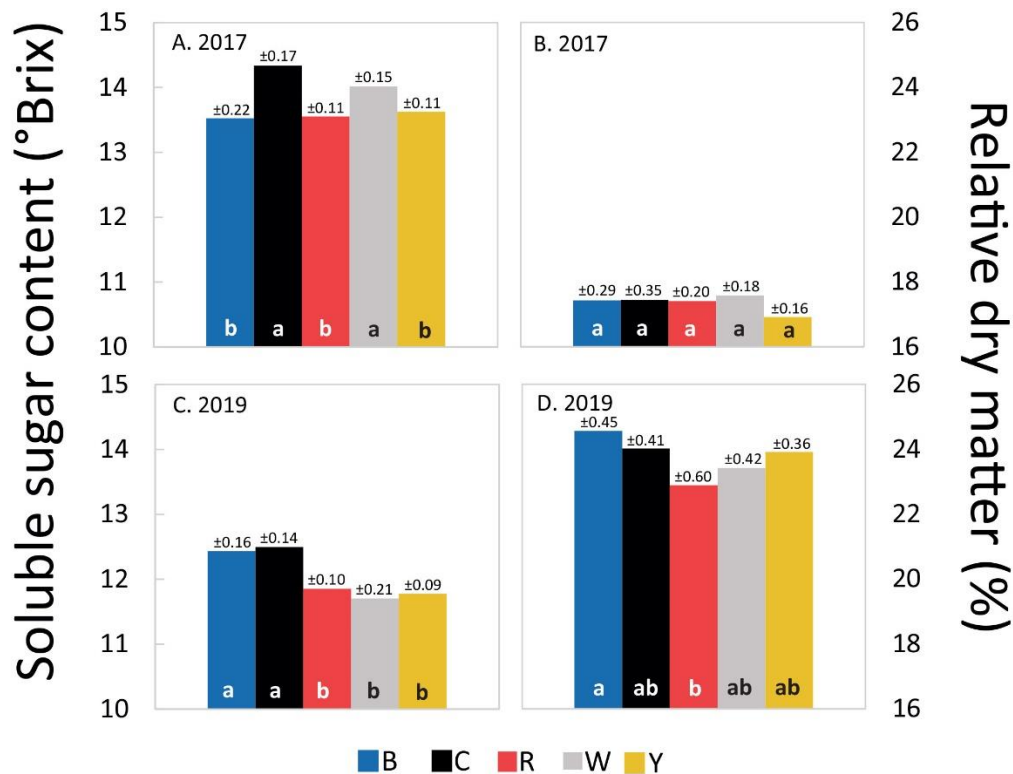


Figure 6. Soluble sugar content and relative dry matter of blue (B), control (C), red (R), white (W) and yellow (Y) LEs. Plots A and B are referred to 2017, while C and D are referred to 2019. Different letters, among columns, indicate significant difference at $P < 0.05$. Numbers on top of each column are standard error values.

3.6. Correlations study. Fruit quality parameters (fruit weight, SSC and RDM), related to fruit growth parameters (AGR_{hour} , mean and total gained weight, AGR_{max} and AGR_{min}), did not show significant relationships in both years (results not shown).

Fruit growth parameters, cited above, related to the crop load in the two seasons were significant only in 2019, when a significant positive correlation was found for AGR (hourly mean) (r 0.90, p 0.04) and total gained weight (r 0.91, p 0.03).

A significant negative relationship between AGR and AER was also found (Fig. 7A, B). In 2017, r values were always above 0.70. The relation between AGR and AER is strictly different in 2019. The r values above 0.70 in C, R and Y highlight the non-randomness of data while this was not true for B and W that had values of 0.52 (B) and 0.62 (W). Nevertheless, p values highlight the significance of the relationships and all treatments had p values below 0.05 except B and R with a p of 0.15 and 0.075, respectively.

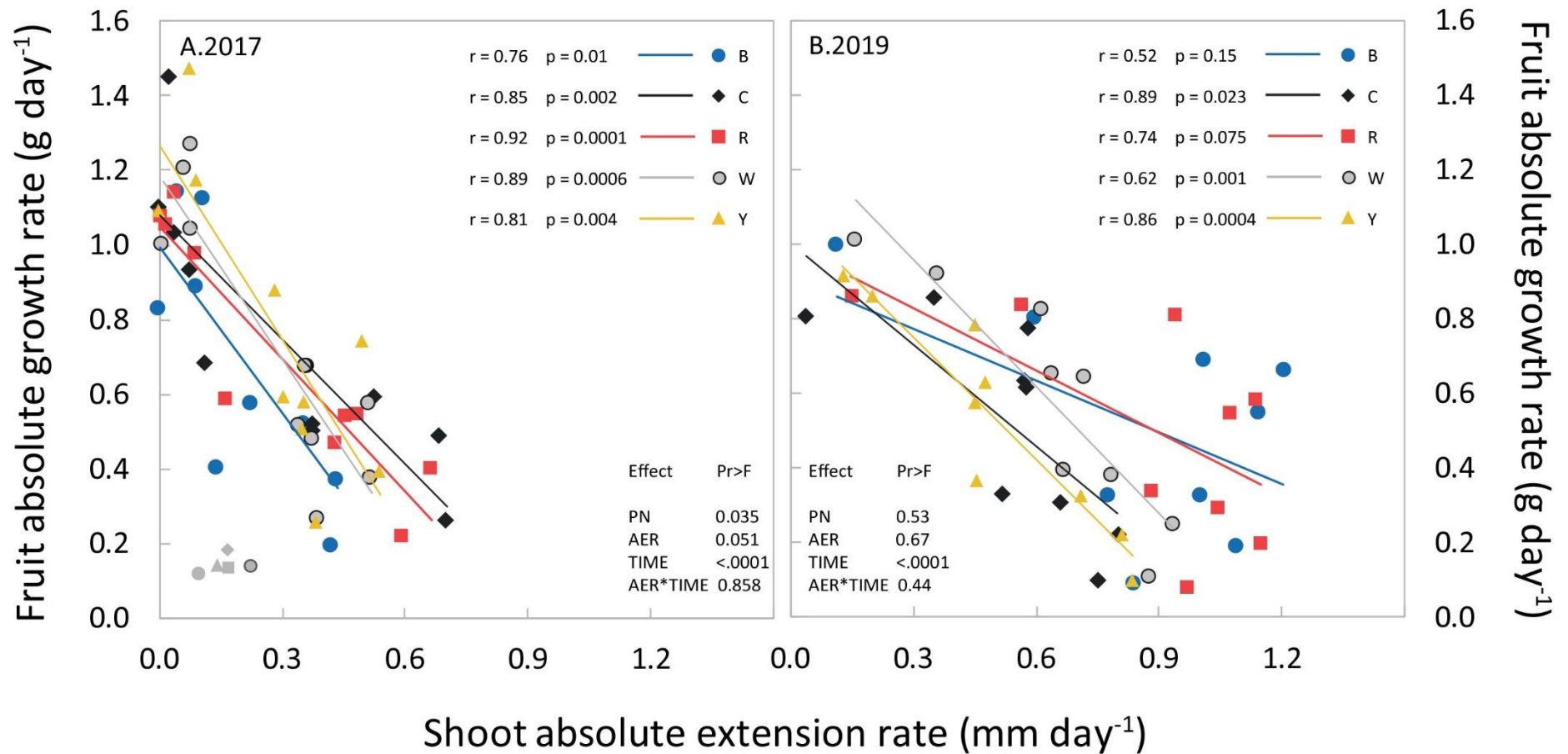


Figure 7. Relationships between shoot absolute extension rate and fruit absolute growth rate obtained with caliper measurements, of each LE, for 2017 (A) and 2019 (B). Each symbol represents a date of measurement, thus the mean of 32 extension shoots and 32 fruit. The effects of LE, AER, TIME and AER*TIME are shown (Pr>F). For each relationship, r and p values are reported. The grey cluster of symbols in the bottom left corner (A) represents a group of outliers.

4. DISCUSSION

One of the objectives of this work was to detect the possibility that coloured nets could modify the early stages of fruit growth, with consequences on final fruit weight. Regardless of the very different crop loads between the two years (Fig. 5A, 5C), average fruit weight seemed to follow a similar behaviour. Higher values were reached for fruit under the W and C LEs (Fig. 5B, 5D), whereas the other LEs were always lower. SSC and RDM results in the two years (Fig. 6), did not report similar trends, except for °Brix, with higher values for C fruit and lower ones for R fruit (Fig. 6A, 6C).

Another objective of this work was to evaluate how photoselective nets can influence fruit growth, during cell division stage. Since technical issues occurred, the discussed data covers the final period of cytokinesis.

In 2017, fruit daily growth at 50 (Fig 4A) and 61 DAFB (Fig 4C) was characterized by shrinkage during the central hours of the day, for all LEs. Shrinkage was more or less pronounced, with no significant differences among treatments (Fig. 4C). The same periods in 2019 showed a similar behaviour. At 56 DAFB (Fig. 4B) no significant differences were present, indicating that LEs were possibly not influencing fruitlet growth enough. Significant differences among LEs were present at 63 DAFB (Fig. 4D), where C fruit tended to be higher for most of the first part of the day. Shrinkage was present, however no differences occurred during the central part of the day.

At 77 DAFB and 91 DAFB a change in fruit growth behaviour took place. In 2017, C and Y LEs did not shrink during the central hours of the day, unlike R and W (Fig. 4E), suggesting that their fruit xylem might be rather dysfunctional as it usually occurs at this time of apple fruit development (Lang, 1990). These differences in the fruit daily growth pattern suggest a transitioning phase for these specific LEs. Unluckily, no significant differences among the four LEs allowed to validate this statement. The lack of significance is probably caused by the very

low number of replicates that were used for this specific period (Fig. 4E). At 91 DAFB (Fig. 4G) all LEs do not present shrinkage.

In the second phases of 2019, from 70 to 90 DAFB (Fig. 4 F-H), all LEs had a marked shrinkage during the central hours of the day, showing a still active xylem. Compared to 2017, mean and total gained weight values are much lower (Table 1). This pronounced difference is probably caused by the weather, in fact spring 2019 (precisely, until 60 DAFB) had lower VPD values, with low temperature and consequently delayed fruit development, causing slow growth and delaying xylem dysfunctionality (Lang, 1990). Another probable cause of delayed xylem closure was the lower incidence of PAR in 2019, which showed lower values compared to 2017. The environmental differences between the two years thus played a major role in dictating fruit growth, as stated by Stampar et al. (2002), regardless of LEs.

A final remark has to be dedicated to W net daily growth, in 2017. Although this treatment had the highest fruit weight at harvest (Fig. 5B), it did not appear to have higher growth rates (Fig. 4A-C-E-G, Table 1) during the initial growth stages. It is believed that the fruit gauges of this specific light environment were installed on fruit that were not representative (i.e. with low growth rates). Moreover, in some cases, appropriate data was not available, leaving down to 3 sensors LE⁻¹. Such a low number of replications in some cases may not be enough to generate significant differences (Fig. 4E), caused by a less dispersion of the statistical error.

Fruit growth during the first part of the season can be modified with the use of photosensitive nets, however, differences occurring at harvest (average fruit weight) may not reflect spring and early summer growth. As literature states (Pretorius et al. 2004; Okello et al. 2015), cell expansion phase is important for determining final fruit weight, since this phenological stage can also be influenced by external factors, such as weather, water availability and light. It has to be underlined that weather and orchard management play a major role in dictating the

development of fruit. Less warm and sunny springs, plus vigorous shoot outburst, slow down reproductive growth (Wünsche and Lakso, 2000), not to mention the effects of crop load (Naor et al. 1997), this latter factor being significant for fruit growth parameters in 2019.

The last objective to analyse, was to understand if coloured nets can modify the competition between vegetative and reproductive growth.

Lakso and Goffinet (2013) noted how, about 3-4 weeks after bloom, the tree urges vegetative growth, to the detriment of the productive yield (Grappadelli et al. 1994). In Table 2, year 2017 showed no significant differences, regardless of LEs, known to impact on vegetative growth (Shahak et al. 2004a, 2004b; Mupambi et al. 2018). In 2019, the situation was completely different, and LEs seemed to influence vegetative growth. A possible cause, of such pronounced growth, could be the heavy pruning, which took place in the former winter. Thereafter, it is possible that the higher crop load (Fig. 5C), along with higher vegetative growth, contributed to lower fruit weight at harvest (Naor et al. 2008; Radivojevic et al. 2012). In, 2019, B and R LEs showed the highest vegetative extension (Table 2), thus shoots were favoured, i.e. they received more photosynthates, with the results of lighter fruit (Fig. 5B-D). On the contrary, in C and Y the translocation towards the fruit was favoured, as confirmed by the average higher fruit weight values (Fig. 5B-D). This is important, because with the different LEs used, under different climatic conditions, different results are expressed that could be interesting to improve vegetative growth, instead of reproductive growth.

The correlations between AGR and AER in 2017 (Fig. 7A) showed differences for all LEs with r values above 0.7 and p -values below 0.05, which highlight the competition between shoot and fruit (Wünsche and Lakso, 2000; Grappadelli et al. 1994). In 2019 (Fig. 7B), C, Y and W have significant relationships between AGR and AER. This underlines that B compared to the other LEs did not impact in the same way on the competition between the two structures, leading to contradicting results. The slope analysis for 2017 (Fig 7A), highlights significant effects of LEs

and only slightly of AER. In 2019 (Fig. 7B), neither LEs nor AER appeared to influence fruit AGR. This difference between the two years is probably due to the different crop load in these seasons, in fact, there was a significant relationship between crop load, AGR_{hourly} and total gained weight, for season 2019 (r values of 0.90 and 0.91). In 2017, there was a lower number of heavier fruits, while in 2019 there was a higher number of fruits with a lower weight. This means that in 2017 the competition for photosynthates between fruit and shoots was lower, pushing more towards the fruit; instead, in 2019 the influence of shoots was markedly higher, caused by the heavier pruning carried out in 2018 which led to a higher number of sprouts.

In both years, among the coloured nets, higher fruit weights were obtained under C and W nets. In the first case, this could be a consequence of the different types of net installation. If coloured nets were installed on individual rows, C net was left open as to cover the rows as the traditional roof system. Thus, this last cover potentially allowed higher incoming radiation to reach the trees. Higher growth values would be due to a higher amount of total light filtering below the C treatment. Therefore, light quantity was more effective than light quality.

In the second case, the W net alternated significantly higher, to intermediate, to lower values of fruit growth, during 2017 and 2019, for all the observed parameters (Fig. 4, Table 1). Surprisingly, it harvested bigger fruit, compared to the other LEs (Fig. 5B,D). This is probably caused by the higher transmittance in the blue light regions (Fig. 2A) and PAR (Fig. 2E) (Bastías, 2011; Kong et al. 2012), that may have modified leaf anatomy, enlarging the stomata (Kong et al. 2012) and consequently improving the photosynthetic performance. Fruit growth parameters measured with fruit gauges did not allow to detect such information, probably due to the low number of replicates which was not enough and/or the selected apples were not representative of the LE.

The Y net was able to favour fruit growth, in 2017, possibly thanks to the higher light scattering properties of this net (Shahak et al. 2016), explaining the higher AGRs (Table 1). Another

possible reason could be the high transmittance in the PAR region (400-700 nm) (Fig. 2E), in fact, this factor added to the high scattering proprieties, may have enriched the light microenvironment (Hemming et al. 2016) enough to justify higher growth rates, although not for the whole season (Fig. 4, Table 1). Consequently, harvest results did not reach high values, as the W ones (Fig. 5B). In 2019, Y net showed lower values for extension shoot and fruit growth rates (Table 1), compared to 2017. Several studies (Dougher and Bugbee, 2001; Kim et al. 2004) give diverse opinions about the impact of this specific wavelength, along with the transmittance of green light. It has been discovered, however, that yellow wavelengths inactivate cryptochromes and increasing green light has been shown to reverse the blue-light-dependent stomata opening response, thus reducing stomatal conductance in many herbaceous crops (Frechilla et al. 2000; Talbott et al. 2002; Kim et al. 2004), with a dose-dependent behaviour. The spectral transmission of the Y net (Fig. 1) could partly explain why it had the lowest growth in 2019, that negatively influenced fruit and shoot growths. Higher transmissions of green and yellow light (Fig. 2B,C), along with lower ones of blue light portions (Fig. 2A) were found, thus potentially explaining the lower tree development. Lower blue light transmission has been proved to affect the development of foliar anatomy (Kong et al. 2012), reducing the mesophyll layer (Pushnik et al. 1987; Saebo et al. 1995). The green light reversal effect and inactivating properties of green-yellow photoreceptors may have further intensified the lower performance of trees under the Y net compared to other treatments.

R net was mostly negative or intermediate, in terms of fruit weight and growth in both seasons (Fig. 5B,D). This LE had the lowest amount of PAR and blue light, compared to the other LEs (Fig. 2) and it is expectable that photosynthetic export may have been directed mainly to the extension shoots, in fact, R shoots had accumulated more growth (Table 2) along with C ones. Under the R net, probably the shade avoidance syndrome was triggered, caused by the lower transmission of PAR and of also by blue light (Boini et al. 2020; Ballare et al. 1991; Pierik et

al. 2004; Keuskamp et al. 2011), which might have been translated in reduced mesophyll layer, altering the leaf anatomy. As a result, photosynthetic performance could have been affected. In 2019, B net appeared to negatively influence fruit growth (Fig. 5D), nevertheless, shoot elongation resulted the highest (Table 2). The effect of pruning had a major influence on AER, thus trees were not affected by the dwarfing effect of this LE. At harvest, however, B fruits were among the smallest ones. It can be assumed that as for R, there was an imbalance of the resources transfer.

It has to be pointed out that the heavy pruning before 2019's trial highly impacted on the orchard's response. There is the possibility that nets shading at around 20% were not effective enough to induce evident differences, or to surpass the intensity of the vegetative outburst of the year 2019. In addition, several variables have not been considered, for example the differences between the internal and external net temperature or the leaf gas exchange, hence limiting the discussion of the results. Mupambi et al. (2018) explain why research that involve light manipulation can give contradictory outcomes.

5. CONCLUSION

Photoselective nets are an important tool that can be used to modify the orchard environment. Their application during the initial phase of fruit development can influence fruit growth and shoot extension, impacting on the competition for carbohydrates.

Although differences occurring at harvest time may not reflect spring and early summer growth (i.e. cell expansion stage was not considered), control and white nets appeared more convenient, producing heavier fruit in both years. The blue and red nets did not give satisfactory results in terms of final fruit weight, thus these nets are not suitable in the apple orchard during cell division. This would apply to some apple producing areas in Italy, such as Southern Tyrol,

which possess environmental restrictions. Finally, yellow nets improved growth in the early stages, however the spectral properties along the season did not help maintaining the same trend, since final fruit weight was not among the highest.

Trees responses were heavily influenced by weather and orchard management, however, the consistent results of final fruit weight, for both years, highlights how the light spectrum had an impact on the development and growth of apple fruit.

Research should try even earlier than 20 DAFB, to manipulate light quality. It is therefore necessary to approach this new technology, with further studies focusing on the differences between each single factor that could improve and extend the use of photosensitive nets.

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