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Light Quality Environment and Photomorphological Responses of Young Olive Trees

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Abstract: Tree densities have increased greatly in olive orchards over the last few decades. In many annual crop species, increased density reduces the horizontal red/far-red (R/FR) and blue/green (B/G) ratios during canopy development even before direct shading occurs, and such changes are known to alter plant morphology. This study with olive trees evaluated: (1) whether the leaf area index (LAI) of neighboring trees modifies the light quality environment prior to a tree being directly shaded and (2) the potential morphological responses of three olive cultivars to changes in light quality. Increasing LAI using different spatial arrangements of potted, three-year-old trees reduced the horizontal R/FR ratio more than that of the B/G ratio. Cultivar-specific responses to low R/FR ratio were observed for individual leaf area and aboveground/belowground biomass ratio using laterally positioned FR mirrors or green fences. No statistically significant responses were detected in response to green vegetation fences that reduced both horizontal R/FR and B/G ratios, but a cluster analysis grouped together the overall morphological responses to both FR mirrors and green fences. These results in olive trees suggest that cultivar differences in response to light quality may be relevant for understanding adaptation to dense orchards and identifying cultivars best suited to them.

Keywords: blue light; Olea europaea L.; red-to-far-red ratio; shade avoidance syndrome; shade tolerance

1. Introduction

Olive production has intensified over the last few decades with a shift from traditional low density orchards towards super high density hedgerow systems [1]. The super high density production model (SHD; 1000 to 2500 trees ha⁻¹) leads to high yields in only a few years after planting and is designed for mechanical harvesting with efficient straddle harvesters [2]. Due to less intra- and inter-row spacing, SHD orchards reach high levels of leaf area index (i.e., m² of leaf area per m² of soil surface) before individual trees cover their allotted space. This leads to a greater interception of photosynthetically active radiation (PAR) early in hedgerow formation [3], and oil yield is maximized when trees have grown enough to intercept about 65% of incident PAR [4,5].

Several studies have addressed the growth and reproductive responses of mature olive trees to PAR using artificial shading. For example, fruit weight and oil concentration were reduced when the percentage of daily PAR received under shade cloth was below 40% [6], while fruit set and vegetative growth were reduced below a slightly higher PAR threshold [7]. Leaf morphological characteristics are also altered by shading, including increased
individual leaf area and decreased leaf thickness within the hedgerows [8,9]. In contrast, responses to light quality in olive orchards have received little or no attention [1,10].

Light spectral composition changes within plant canopies because leaves and other green tissues differentially absorb or reflect specific wavelengths [11]. Green leaves strongly absorb radiation in the red (R) and blue (B) with weak absorption in the green (G) and far-red (FR) wavelengths. Thus, as plant density and LAI increase, sharp reductions occur in the B and R compared with the G and FR [12,13]. Changes in light spectral composition can also be observed before any direct shading from foliage because light reflected by neighboring plants is high in the G and the FR wavelengths [14]. The associated reduction in the red-to-far-red ratio (R/FR) reflected by neighboring plants can be used by a plant as a signal to indicate impending light competition [15].

Plant morphology in annual crop species often responds to changes in the light spectral composition (reviewed by [16]). Many annual plants detect changes in R/FR and B/G ratios with photoreceptors (i.e., phytochromes and cryptochromes) that trigger shade avoidance responses before shading occurs (reviewed by [17]). For example, reductions in the R/FR ratio of light prior to direct shading were associated with increases in internode and petiole length in *Nicotiana tabacum* and *Arabidopsis thaliana*, respectively [18,19]. Such responses contribute to greater new leaf formation in the upper canopy where light intensity is high in order to avoid shading, and they may affect biomass partitioning between organs and crop yield [20].

Few studies have evaluated the responses to light quality manipulations in woody perennial species including fruit trees [11,21]. Increases in main stem and shoot growth were observed in peach and cherry trees when grown under photo-selective films that reduced R/FR [22]. Shoot growth was also reduced in Scots pine when illuminated by laterally positioned light sources that reduced R/FR without affecting PAR. However, grape vine (*Vitis vinifera* L.) showed no increase in stem elongation or number of ramifications when plants were exposed to low R/FR ratio using lateral illumination with FR LEDs [23]. Additionally, the limited information available suggests a wide range of leaf area response to low R/FR in woody species [23–25]. To the best of our knowledge, olive tree morphological and biomass partitioning responses to changes in light quality have not yet been evaluated. Such responses could ultimately be of agricultural importance if they affect hedgerow structure in SHD olive orchards [1].

In addition to interspecific differences, photomorphogenic responses may also vary within the same species [26,27]. For example, Botto [28] found that *Arabidopsis thaliana* plants from coastal populations of northeast Spain showed a reduction in aerial biomass when illuminated laterally with FR to mimic neighboring plants, while mountain populations did not respond to changes in R/FR. Additionally, different responses to low R/FR were observed between soybean cultivars with increasing internode length occurring in cvs. Sultana and Merlin and no response occurring in cv. Lissabon [29]. Given the high number of genetically diverse olive cultivars, different photomorphogenic responses to light quality may exist. The objectives of the present study were to: (1) evaluate whether the leaf area index (LAI; m² of leaf area per m² of soil surface) of neighboring olive trees modifies the light quality environment (R/FR and B/G ratios) prior to a tree being directly shaded and (2) assess the potential morphological responses of three olive cultivars (Arbequina, Coratina, Arauco) to changes in light quality that simulated the presence of neighboring trees.

2. Materials and Methods

The study was performed at the experimental field station of CRILAR-CONICET in the province of La Rioja in northwestern Argentina (28°48′ S, 66°56′ W; 1325 masl) during the 2018–2019 growing season. The field station is located near the Andes mountains, and the region is hot and dry with an annual precipitation of 100–150 mm and annual evapotranspiration of about 1600 mm [30,31].
2.1. Light Quality Response to Leaf Area Index of Neighboring Trees

Horizontally oriented radiation measurements were performed in February 2019 using potted trees arranged in different densities to create a wide range of LAI from neighboring trees. Nine three-year-old olive trees (cv. Coratina) growing in 30 L pots with an average leaf area of 0.61 m$^2$ tree$^{-1}$ were used. Leaf area per tree was determined after the radiation measurements were done by counting the total leaf number of three trees and multiplying leaf number per tree by average individual leaf area. Individual leaf area was obtained by dividing the weight of 150 randomly collected leaves (i.e., 50 from each tree) by the specific leaf mass (mg cm$^{-2}$) of the same leaves, which was estimated from leaf disks of known area. The arrangements included a 4 m $\times$ 1.3 m spacing to simulate a recently planted SHD orchard and denser tree spacings of 1.5 $\times$ 1.5, 0.75 $\times$ 0.75, 1.0 $\times$ 1.0, and 0.5 m $\times$ 0.5 m to emulate what would occur as the tree crowns grew. In all cases, the rows of trees were arranged in a N–S orientation. The range of LAI generated was 0.2 to 2.4 m$^2$ of leaf area per m$^2$ of ground area (Figure 1A,B). LAI values of 2.5 m$^2$ m$^{-2}$ have recently been reported for four-year-old SHD orchards [32].

The light environment was characterized for the central tree of each arrangement at R (660 nm) and FR (730 nm) wavelengths with a Red/Far-Red sensor (µmol m$^{-2}$ s$^{-1}$; SKR 110,
Skye Instruments Ltd., Llandrindod Wells, UK). Blue (465 nm) and G (525 nm) wavelengths were evaluated with a RGB sensor (µW cm⁻²; APDS-9960, Avago Technologies Ltd., San José, CA, USA) connected to an Arduino board (MEGA 2560 Compatible-CH340; Todo Micro, Buenos Aires, Argentina). The sensor heads were positioned at a distance of 5 cm away from the exterior of the central tree crown and oriented perpendicular to the crown at three heights in order to measure horizontal changes in light quality associated with the presence of the neighboring trees. The chosen crown heights of 0.5, 1.0, and 1.5 m represented the lower, middle, and upper portions of the crown, respectively (Figure 1C). The measurements were performed at four azimuthal orientations (N, S, E, and W) and at five times along the course of the day (8:00, 10:00, 12:00, 14:00, and 16:00 h solar time) for all of the tree arrangements. All measurements were made on three consecutive sunny days, with each day considered as a repetition.

2.2. Morphological Responses to Light Quality

2.2.1. Plant Material

Young plants of cultivars Arbequina, Coratina, and Arauco were obtained from the nursery of the Junín Experimental Station (Mendoza Province) of the Instituto Nacional de Tecnología Agropecuaria (INTA). The cv. Arbequina is widely used in SHD orchards worldwide due to its compact growth form and is originally from Catalonia, Spain. The cv. Coratina is mainly used in lower density orchards due to its often greater vigor and is originally from Apulia, Italy. The cv. Arauco, is also used in lower density orchards and is the only Argentine cultivar recognized by the International Olive Oil Council (2000) [33].

The experiment was conducted from 1 October 2018 (mid-spring) to 15 February 2019 (mid-summer) at the experimental field station of CRILAR-CONICET in La Rioja. The plants were seven months old at the beginning of the experiment and were grown in 700 cm³ plastic pots filled with a sand/peat/perlite mixture (1:1:1). The young plants were well-watered twice a day through a microtube spider-type irrigation system based on the daily atmospheric demand. They were fertilized manually with 2.5 g macronutrients (15 N: 15 P: 15 K) every 15 days. To reduce light reflection from the soil, the pots were placed on six 40 cm high and 6 m long wooden tables oriented E–W.

2.2.2. Experimental Design and Light Quality Treatments

There were two light quality treatments (i.e., FR mirrors and green fences) with each treatment having its own control (i.e., control mirrors and control fences) (Figure 2A). We used one FR mirror per plot that selectively reflected FR wavelengths horizontally to reduce the R/FR ratio received by the young plants (Figure 2B) and one green vegetation fence (GF) per plot that reduced both the horizontal R/FR and B/G received by the olive plants. One plant of each cultivar was placed to the N of each mirror or fence for a total of three plants per plot. Thus, the experimental design was a split-plot design with light quality as the main plot (FR mirror or GF) and the cultivar as the subplot. Ten replicate plots were used for each of the light quality treatments and controls, and one plant of each of the three cultivars was randomly assigned to each plot. The plants were separated by 20 cm within a light quality plot, and plots were separated by 35 cm.

The FR mirrors were built using cardboard sheets (30 cm height × 60 cm length and 4 mm thick) that were first covered by aluminum foil, then by a red acetate layer (0.2 mm, LEE filters 106 Primary Red, Central de Lamps, Buenos Aires, Argentina), and lastly by a blue acrylic layer (2 mm, 2031, Paolini SAIC; Buenos Aires Argentina) [34]. These layers covered the entire cardboard surface. The control mirror had a neutral wavelength reflection and consisted of a similar cardboard sheet covered with black polyethylene (200 µm). All mirrors were placed 20 cm to the S of the young olive plants, given that the sun at midday is positioned to the north in the southern hemisphere, and the height of the mirrors was adjusted to account for plant growth with the main stem apex coinciding with the center of the mirror. The mirrors were inclined 30° from vertical to provide maximum reflection for the midday solar azimuth angle at our latitude.
Figure 2. Field arrangement of the two light quality treatments and their controls (A). Horizontal rectangles represent the plots of the far-red mirror (red rectangles) and green fence (green rectangles) treatments as well as plots with control mirrors (red-bordered rectangles) and control fences (green-bordered rectangles). Vertical lines represent one individual plant of each cultivar per plot. Photograph showing three plants in front of a far-red mirror (B).

The GF closely simulated the modifications in the light environment that occur due to the reflection of light by neighboring green plants [14]. The GF were formed using pots containing a 40 cm height wheat–rye mixture placed 20 cm to the S of the olive plants. At the start of leaf senescence of the wheat–rye mixture, it was replaced by a fence made of clades of cactus (Opuntia ficus-indica) on 3 January 2019. The control fence (CF) was composed of dried rush (Juncus imbricatus). Similar to the mirrors, both GF and CF fences were positioned such that the main stem apex of the trees coincided with the center of the fence.

2.2.3. Light, Temperature, and Relative Humidity Measurements

The light horizontally reflected by the treatments and controls was evaluated in all of the experimental plots three times a day on 20 November 2018 (9:00, 12:00, and 15:00 h solar time) at the R, FR, B, and G wavelengths. The Red/Far-Red and the RGB sensors were placed such that the sensor head was placed horizontally with the sensor window facing the mirror or the fence in order to measure horizontal changes in light quality reflected by the mirrors or fences at stem apex height. Vertical light fluxes were also measured at midday on the apex of the central plant of each plot. The vertically and horizontally reflected PAR were estimated using previously constructed relationships between R, B, and G fluxes and values from a PAR sensor. Humidity and air temperature were measured at the same times using a digital thermohygrometer (Hygropalm 2, Rotronic Ag, Hauppauge, NY, USA).

More detailed, hourly measurements (5:00 to 19:00 h; solar time) of the R/FR ratio were also evaluated in one randomly-selected FR mirror plot and one control mirror plot. The mirrors were effective in decreasing the R/FR ratio in relation to the control between 5:00 and 15:00 h (average R/FR = 0.44 for the FR mirror versus 1.10 for the control). From 16:00 to 19:00 h, no differences between the treatments were observed in the R/FR ratio due to the sun falling behind the mountains to the west of the experimental station.

2.2.4. Morphological and Biomass Measurements

The initial size of all young olive plants, including main stem length, number of nodes, and number of leaves, was evaluated at the beginning of the experiment. The initial size of the plants assigned to the two treatments and their controls was similar for all variables. At the end of the experiment, main stem elongation was calculated as the difference between stem length at the beginning and the end of the experiment. The number of nodes per main stem that were formed during the experiment were also counted, and the internode length was estimated by dividing stem elongation by the number of new nodes.

Leaf length and width were measured at the end of the experiment on five fully developed leaves that formed during the latter part of the experiment. Individual leaf area of the same five leaves was determined by sampling leaf disks of known area from them and drying the disks and the leaves in a forced-air oven at 70 °C. The individual leaf area
was then calculated by dividing leaf dry weight by the specific leaf mass (mg cm$^{-2}$; SLM). The increase in leaf number per plant was calculated by counting the number of leaves at the beginning and the end of the experiment. Additionally, the increase in plant leaf area was estimated by multiplying the increase in the number of leaves per plant by the individual leaf area. The number of axillary shoots per plant was counted only at the end of the experiment since few plants had axillary shoots at the beginning of the experiment. Axillary shoot angles were estimated using digital photographs (Leica 13 MP dual camera, Huawei Vns L23, Huawei Technologies Co., Ltd., Longgang District, Shenzhen, China) that were processed using ImageJ software (ImageJ, U.S. National Institutes of Health, https://imagej.nih.gov/ij/, 1997–2018 (accessed on 10 June 2019)).

Each plant was harvested at the end of the experiment to determine biomass. Leaves, stems, and roots from each plant were separated and dried in a forced-air oven at 70 °C until reaching a constant dry weight. Total plant biomass and aboveground/belowground biomass ratio were also calculated.

2.3. Statistical Analyses

For the light characterization experiment, analysis of variance (ANOVA) was used to assess the potential effects of LAI and azimuth orientation on R/FR and B/G ratio. A split-plot ANOVA was performed separately for each of the two light quality treatments (FR mirror and green fence) and its respective control to assess the effects of light quality in the different cultivars on the morphological and biomass variables. In all cases, the Fisher LSD post-test was used to detect the significance of differences between means ($p < 0.05$). A hierarchical clustering analysis by average linkage (Euclidean distance) was carried out to group light quality treatments and controls based on their mean values of morphological and biomass variables. A principal components analysis was also performed to further identify potential patterns due to cultivar and light quality for these same variables. The statistical analyses were performed with InfoStat statistical software [35].

3. Results

3.1. Light Quality Characterization in Response to Leaf Area Index

Increasing LAI from 0.2 to 2.4 m$^2$ m$^{-2}$ led to decreases in the R/FR ratios from 0.84 to 0.56 when averaged over the different azimuth orientations and crown heights along the course of the day (Figure 3A). In contrast, the B/G ratio decreased from only 0.81 to 0.76 (Figure 3B), although this decrease was still statistically significant ($p < 0.05$).

The R/FR and B/G ratios varied over the course of the day by azimuth orientation for the intermediate LAI evaluated (1.1 m$^2$ m$^{-2}$; Figure 4). In the early morning (8:00 solar time), the horizontally oriented R/FR ratio of light incident on the crown was highest on the E side of the central tree in the array, as would be expected based on the position of the sun with the R/FR ratio being intermediate on the N side and lowest to the S and W ($p < 0.05$; Figure 4A). At mid-morning (10:00), the horizontal R/FR ratio was high on both the E and N sides and low on the S and W sides. At midday, no statistically significant differences in R/FR between azimuth directions were observed. In mid-afternoon (14:00), the R/FR ratio was highest on the W side, intermediate on the N side, and low on the E and S sides ($p < 0.05$). In the late-afternoon, the values on the W side were higher than those of the other azimuth orientations as would be expected.

The B/G ratio did not show large differences between azimuth orientations over the day (Figure 4B). On all sides of the tree, the ratios increased from 8:00 to 10:00 h and showed a tendency to decrease during the afternoon. The only statistically significant ($p < 0.05$) lower B/G ratio with respect to the other orientations was detected on the N side in the early afternoon (14:00).
At mid-morning (10:00), the horizontal R/FR ratio was high on both the E and N sides and low on the S and W sides. At midday, no statistically significant differences in R/FR between azimuth directions were observed. In mid-afternoon (14:00), the R/FR ratio was highest on the W side, intermediate on the N side, and low on the E and S sides. In the late-afternoon, the values on the W side were higher than those of the other azimuth orientations as would be expected.

The B/G ratio did not show large differences between azimuth orientations over the day. On all sides of the tree, the ratios increased from 8:00 to 10:00 h and showed a tendency to decrease during the afternoon. The only statistically significant lower B/G ratio with respect to the other orientations was detected on the N side in the early afternoon (14:00).

**Figure 3.** The red/far-red (R/FR; (A)) and blue/green (B/G; (B)) ratios of horizontally reflected light as a function of leaf area index (LAI, m² leaf area m⁻² ground surface) of neighboring trees. Symbols represent the averages ± SE of the measurements performed at four azimuthal orientations and three crown heights over the course of the day on three sunny days (n = 3). Different letters indicate significant differences in the R/FR ratio or B/G ratio between LAI values using the Fisher LSD post-test (p < 0.05).

**Figure 4.** Diurnal dynamics of horizontally reflected red/far-red (R/FR; (A)) and blue/green (B/G; (B)) ratios measured at different azimuthal orientations (N, S, E, and W) for an intermediate leaf area index (1.1 m² m⁻²). The symbols represent the averages ± SE of the three measurement dates (n = 3). Asterisks indicate significant differences between orientations for a given solar time using the Fisher LSD post-test (p < 0.05).
3.2. Morphological Responses to Light Quality

3.2.1. Light Quality Characterization of Far-Red Mirrors and Green Fences

The R/FR ratio at 9:00 and 12:00 h was reduced from about 0.9 with the control mirror to less than 0.4 with the FR mirror (Figure 5A). At 15:00 h, the difference was smaller with values of about 1.05 for the control and 0.85 for the FR mirror. The GF also significantly reduced the R/FR ratio at 9:00 and 12:00 h, with the CF having ratios of about 0.75 and the GF less than 0.4 (Figure 5B). However, no difference was apparent between the CF and the GF at 15:00 h.

![Graphs showing R/FR and B/G ratios](image)

Figure 5. Red/far-red (R/FR) and blue/green (B/G) ratios of the horizontally reflected light incident on the main stem apex of plants receiving reduced R/FR and B/G using FR mirrors (A,C) or green vegetation fences (B,D). Radiation measurements in R, FR, B, and G wavelengths were made with the sensors oriented perpendicular to the mirror or fence in order to measure horizontal changes in light quality reflected by the mirrors. Symbols represent the averages ± SE (n = 10). Asterisks indicate significant differences between a light quality treatment and its control for a given solar time using the Fisher LSD post-test (p < 0.05).

The B/G ratio was slightly higher with the FR mirror than the control at 9:00 and 12:00 h (Figure 5C) due to slightly lower reflection in the G by the FR mirrors. No difference was detected at 15:00 h. Larger differences between the GF and CF were observed at all three solar times with lower average B/G values in GF (0.80) than in CF (0.85) (Figure 5D).

The PAR received horizontally from the FR mirrors, the GF, and their controls had a very narrow range (123–136 µmol m⁻² s⁻¹) when measured at midday, with no statistically significant differences between them. This PAR was about 6% of the PAR received vertically (2040 µmol m⁻² s⁻¹). Additionally, there were no significant differences in air temperature or relative humidity between any of the treatments at any time of measurement (data not shown).

3.2.2. Morphological Responses to Far-Red Mirrors

Leaf area response to the FR mirrors differed between the cultivars. While individual leaf area was reduced 26% by the FR mirrors in cv. Arbequina (p < 0.05), an increase of
18% was observed in cv. Arauco ($p < 0.05$), and no response was apparent in cv. Coratina (Figure 6A). These changes in leaf area were primarily attributed to differences in leaf width and not to differences in leaf length between treatments (data not shown). The increase in leaf number per plant during the experiment was also reduced 16% by the FR mirrors in cv. Arbequina ($p < 0.05$) but was not affected in the cvs. Coratina and Arauco (Figure 6B). The reductions in both individual leaf area and the increase in leaf number per plant in cv. Arbequina led to a 38% decrease in the leaf area produced per plant during the experiment ($p < 0.05$; Figure 6C). No significant effect on leaf area per plant was apparent for the cvs. Coratina and Arauco.

![Figure 6](image_url)

**Figure 6.** Individual leaf area (A) and the increase in leaf number (B) and in leaf area (C) per plant for the experiment with far-red or control mirrors. Bars represent averages ± SE ($n = 10$). Different letters indicate significant differences between the means for a given morphological variable using the Fisher LSD post-test ($p < 0.05$).

Main stem length more than doubled during the experiment, but no statistically significant differences in stem elongation in response to the FR mirrors were detected in any of the three cultivars evaluated (Table 1). However, internode length was 30% lower with the FR mirrors than with the control mirrors in cv. Coratina ($p < 0.05$), while the
increase in the number of main stem nodes was reduced by 18% in response to the FR mirrors in cv. Arbequina.

Table 1. Stem growth responses to far-red (FR) or control mirrors in three olive cultivars. Average values ± SE (n = 10) are shown. Different letters indicate a significant difference between the means for a given morphological variable using a Fisher LSD post-test (p < 0.05).

| Variables                        | Cultivar  |          |          |          |          |          |
|----------------------------------|-----------|----------|----------|----------|----------|----------|
|                                  | Arbequina | Coratina | Arbequina | Coratina | Arbequina | Coratina |
| Main stem elongation (cm)        | 22.9 ± 3.2b | 29.5 ± 3.4c | 18.7 ± 3.2a | 22.8 ± 3.2bc | 18.4 ± 3.8ab | 12.3 ± 3.6a |
| Internode length (cm)            | 3.5 ± 0.4abc | 3.7 ± 0.5bc | 3.1 ± 0.4ab | 4.4 ± 0.4c | 3.2 ± 0.5abc | 2.2 ± 0.5a |
| Increase in main stem nodes (#)  | 6.6 ± 0.5b | 8.0 ± 0.5c | 5.9 ± 0.5ab | 5.0 ± 0.5a | 6.0 ± 0.5ab | 5.8 ± 0.5ab |
| Axillary shoots per plant (#)    | 3.2 ± 0.5bc | 4.3 ± 0.5c | 0.5 ± 0.5a | 0.4 ± 0.5a | 1.9 ± 0.6ab | 1.6 ± 0.6a |
| Axillary shoot angle (°)         | 61.0 ± 3.3b | 56.4 ± 3.6ab | 52.2 ± 3.3ab | 49.8 ± 3.3a | 53.1 ± 4.1a | 53.9 ± 3.8ab |

The leaf biomass per plant was lower with the FR mirrors than with the control in cvs. Arbequina and Coratina (p < 0.05) and was not affected in cv. Arauco (Table 2). Stem biomass also tended to be lower due to the FR mirrors (p < 0.10) in cv. Arbequina. Consistent with the leaf and stem biomass responses, aboveground biomass was 25% and 36% lower with the FR mirrors in cvs. Arbequina and Coratina, respectively, but was not affected in cv. Arauco (Table 2). Since root weight was not different with the FR mirrors in the cvs. Arbequina or Coratina, the aboveground/belowground ratio decreased with the FR mirrors in these cultivars (p < 0.05). Total plant biomass decreased significantly only in cv. Coratina (p < 0.05). While no biomass determinations were made prior to the experiment, the number of leaves quadrupled on average during the experiment, which suggests that most aboveground biomass accumulated during the experimental period.

Table 2. Biomass responses to far-red (FR) or control mirrors in three olive cultivars. Average values ± SE (n = 10) are shown. Different letters indicate a significant difference between the means for a given biomass variable using a Fisher LSD post-test (p < 0.05).

| Variables          | Cultivar  |          |          |          |          |          |
|--------------------|-----------|----------|----------|----------|----------|----------|
|                    | Arbequina | Coratina | Arbequina | Coratina | Arbequina | Coratina |
| Leaves (g)         | 4.7 ± 0.6b | 6.6 ± 0.6c | 2.9 ± 0.6a | 5.4 ± 0.6bc | 4.3 ± 0.7ab | 4.5 ± 0.7ab |
| Stems (g)          | 3.4 ± 0.3ab | 4.2 ± 0.3b | 2.9 ± 0.3a | 3.6 ± 0.3ab | 2.7 ± 0.4a | 2.7 ± 0.3a |
| Roots (g)          | 5.8 ± 0.7a | 5.3 ± 0.7a | 5.6 ± 0.7a | 6.1 ± 0.8a | 5.3 ± 0.8a | 4.7 ± 0.8a |
| Aboveground (g)    | 8.1 ± 0.8ab | 10.8 ± 0.9c | 5.8 ± 0.8a | 9.1 ± 0.8bc | 7.1 ± 1.0ab | 7.3 ± 0.9ab |
| Aboveground/belowground | 1.5 ± 0.2ab | 2.1 ± 0.2c | 1.1 ± 0.2a | 1.6 ± 0.2bc | 1.4 ± 0.2ab | 1.6 ± 0.2bc |
| Total plant (g)    | 13.9 ± 1.3abc | 16.0 ± 1.4c | 11.5 ± 1.3a | 15.1 ± 1.3bc | 12.3 ± 1.6ab | 12.0 ± 1.5ab |
| Specific leaf mass (mg cm⁻²) | 22.7 ± 0.8a | 24.9 ± 0.8a | 23.50 ± 0.8a | 24.7 ± 0.8a | 22.8 ± 0.9a | 22.6 ± 0.9a |

3.2.3. Morphological Responses to Green Fences

Individual leaf area as well as the increase in leaf number and in leaf area per plant during the experiment was similar between GF and CF in all cultivars (Figure 7). The cv. Arbequina had smaller leaves than cv. Coratina (Figure 7A), but it had a greater number of leaves than the other two cultivars (Figure 7B). The increase in leaf area per plant during the experiment was greater in cv. Arbequina than in cv. Arauco, with intermediate values in cv. Coratina (Figure 7C).
The main stem elongation and other stem-related variables did not show any significant differences between the GF and CF for the three cultivars evaluated (Table 3). There were also no differences between GF and CF in plant biomass or its partitioning (Table 4). Among cultivars, cv. Arbequina had a greater increase in the main stem nodes and axillary shoots per plant, and cv. Arauco had about 40% less stem weight, aboveground biomass, and total biomass (p < 0.05).
Table 3. Stem growth with green (GF) and control fences in three olive cultivars. Average values ± SE (n = 10) are shown. Different letters indicate a significant difference between the means for a given morphological variable using Fisher LSD post-test (p < 0.05).

| Variables                        | Cultivar       | Arbequina | Control | Coratina | GF   | Control | Arauco | GF   | Control |
|----------------------------------|----------------|-----------|---------|----------|------|---------|--------|------|---------|
| Main stem elongation (cm)        |                | 27.3 ± 3.6 b | 29.2 ± 3.4 b | 22.4 ± 3.4 ab | 25.5 ± 3.4 | 12.5 ± 3.8 a | 14.4 ± 3.8 a |
| Internode length (cm)            |                | 3.8 ± 0.7 ab | 3.7 ± 0.7 ab | 3.7 ± 0.7 ab | 5.1 ± 0.7 b | 2.8 ± 0.8 a | 3.1 ± 0.8 ab |
| Increase in main stem nodes (#)  |                | 7.7 ± 0.7 b | 7.9 ± 0.6 b | 5.7 ± 0.6 a | 5.3 ± 0.6 a | 6.3 ± 0.8 ab | 5.5 ± 0.7 a |
| Axillary shoots per plant (#)    |                | 4.3 ± 0.6 c | 3.9 ± 0.6 bc | 1.1 ± 0.5 a | 1.0 ± 0.5 a | 2.3 ± 0.8 ab | 2.8 ± 0.7 abc |
| Axillary shoot angle (°)         |                | 58.1 ± 4.0 a | 59.2 ± 3.8 a | 49.9 ± 3.8 a | 55.1 ± 3.8 a | 53.0 ± 4.2 a | 59.8 ± 4.2 a |

Table 4. Biomass with green (GF) and control fences in three olive cultivars. Average values ± SE (n = 10) are shown. Different letters indicate a significant difference between means for a given biomass variable using a Fisher LSD post-test (p < 0.05).

| Variables                        | Cultivar       | Arbequina | Control | Coratina | GF   | Control | Arauco | GF   | Control |
|----------------------------------|----------------|-----------|---------|----------|------|---------|--------|------|---------|
| Leaves (g)                       |                | 5.6 ± 0.7 bcd | 6.9 ± 0.7 d | 4.6 ± 0.6 abc | 6.2 ± 0.7 cd | 3.1 ± 0.7 a | 3.9 ± 0.7 ab |
| Stems (g)                        |                | 4.1 ± 0.4 b | 4.0 ± 0.4 b | 4.1 ± 0.4 b | 4.6 ± 0.4 b | 2.1 ± 0.4 a | 2.4 ± 0.4 a |
| Roots (g)                        |                | 5.9 ± 0.8 ab | 5.5 ± 0.8 a | 6.5 ± 0.8 ab | 8.1 ± 0.8 b | 4.3 ± 0.9 a | 4.3 ± 0.9 a |
| Aboveground (g)                  |                | 9.7 ± 1.1 c | 10.9 ± 1.0 c | 8.7 ± 1.0 bc | 10.8 ± 1.0 c | 5.2 ± 1.1 a | 6.3 ± 1.1 ab |
| Aboveground/belowground          |                | 1.7 ± 0.2 ab | 2.2 ± 0.2 b | 1.4 ± 0.2 a | 1.5 ± 0.2 a | 1.4 ± 0.3 a | 1.5 ± 0.3 a |
| Total plant (g)                  |                | 15.6 ± 1.7 bc | 16.4 ± 1.6 c | 15.2 ± 1.6 bc | 18.9 ± 1.6 c | 9.5 ± 1.8 a | 10.6 ± 1.8 ab |
| Specific leaf mass (mg cm⁻²)     |                | 24.1 ± 0.8 a | 23.9 ± 0.8 a | 23.5 ± 0.8 a | 23.5 ± 0.8 a | 23.8 ± 0.8 a | 21.9 ± 0.8 a |

3.2.4. Cluster and Principal Component Analysis

A hierarchical clustering analysis integrated morphological and biomass variables in response to the FR mirrors, green fences, and their respective controls for the three olive cultivars (Figure 8A).

Young olive plants exposed to the FR mirror and GF were grouped in one cluster, and both controls were grouped in another cluster. Principal component analysis explained 82% of the total variability, with the first principal component (PC1) explaining 65% and the second principal component (PC2) explaining 17%. PC1 was associated mainly with the increase in leaf area per plant and aboveground/belowground biomass as well as increase in leaf number and leaf weight per plant. Plant biomass, SLM, and the increase in the number of main stem nodes had a smaller contribution to PC1. The PC2 was associated to a large extent with individual leaf area. The PCA highlighted the fact that the three cultivars showed different responses to light quality. In cv. Arbequina, plants with the control mirror and control fence treatments were strongly associated with PC1. In contrast, the GF plants showed less association with PC1, and the FR mirror plants appeared to be negatively associated with PC2. In cv. Coratina, the control plants had some association with PC2, but the FR mirror plants were negatively associated with PC1. In cv. Arauco, little difference was observed between the controls and the treatments.
Figure 8. Cluster (A) and principal component (B) analyses of plant morphological and biomass variable responses to far-red (FR) mirrors, green fences (GF), and their respective controls (C or CF). The different cultivars are abbreviated as cv. Arbequina (Arb), Coratina (Cor), and Arauco (Ara). The variables used in the analyses were individual leaf area, total plant biomass, specific leaf mass (SLM), leaf weight, leaf area per plant, aboveground/belowground biomass (A/B), leaf number per plant, and number of nodes per main stem.

4. Discussion

The influence of plant density on light quality has been well documented in many annual crop species [29,36,37], but little or no information is available for fruit tree orchards [1,38]. The results of our study show that the optical properties of olive leaves can modify the light quality reflected by the tree crown even before direct shading between trees occurs (Figures 3 and 4). Increasing tree density and the corresponding increases in the LAI decreased the horizontally reflected R/FR and B/G ratios. The lowest R/FR (0.56) and B/G (0.76) values were obtained at the highest measured LAI value (2.4 m² m⁻²). Similar LAI values were found cv. Arbequina SHD orchards with a tree density of 1480 trees ha⁻¹ [32]. Thus, changes in the horizontally reflected light quality environment due to neighboring trees can be expected in SHD orchards even before direct shading between trees begins.

Olive leaves have species-specific characteristics that should affect the degree to which light quality is affected in dense orchards. Evidence indicates that the upper leaves of olive trees are more vertically oriented than lower leaves, which has the effect of reducing the...
solar radiation absorbed by the upper leaves and reducing their heat load [39,40]. Fairly vertical leaf inclination angles would lead to considerable reflection of light by the dense layer of trichomes on the partially exposed, abaxial side of the leaf [41]. Given that the spectral distribution of light reflected from the whitish trichome layer should be more wavelength neutral than light reflected by green tissue, the B/G and R/FR ratios would be affected. These optical properties would explain the smaller decreases in the horizontal B/G and R/FR ratios with increasing LAI in our study relative to stands of annual species such as *Datura ferox* and *Sinapis alba* [42].

Individual leaf area responses to low R/FR can be highly variable between species, including both inhibition and promotion of leaf expansion (as reviewed by [43]), and differences between agronomic cultivars have also been observed [29]. In our study, different leaf area responses were found between olive cultivars, with individual leaf area being reduced in cv. Arbequina and increased in cv. Arauco when plants received low R/FR using laterally positioned FR mirrors (Figure 6). This may be related to the considerable genetic differences among olive cultivars in vegetative vigor and growth habit [33,44] that arose during olive domestication around the Mediterranean Basin [45]. The reduction in individual leaf area observed for cv. Arbequina in response to low R/FR prior to direct shading could affect hedgerow structure and improve its performance under SHD by increasing light penetration within the canopy [41]. The reduction in leaf area of cv. Arbequina due to low R/FR does appear to contrast with the larger and thinner leaves of this cultivar that develop within the shade of the tree crown where both PAR and R/FR ratio are low [46]. However, preliminary results evaluating the separate effects of FR supplementation and reduced PAR are consistent with horizontal FR light reducing individual leaf area in cv. Arbequina [47].

In many plant species, early R/FR signals due to the presence of neighbors trigger shade avoidance responses, such as an increase in internode length and stem elongation (reviewed by [17]). These morphological responses are advantageous for survival in high density stands in natural ecosystems, but they may have negative consequences in crop systems because yield can be reduced due to greater biomass partitioning to stems rather than to reproductive organs [20,37]. In woody plant species, increased stem elongation (i.e., shade avoidance) has been reported in some species such as silver birch [48], but no response (i.e., shade tolerance) was apparent in grape vine [23]. In our study, main stem elongation as well as branch number was not affected when the R/FR ratio was reduced using FR mirrors in the three olive cultivars evaluated (Table 1). Internode length was actually 30% lower in plants with FR mirrors in cv. Coratina and was not affected in the cvs. Arbequina and Arauco (Table 1). These stem results suggest that olive trees are shade-tolerant in that they do not attempt to escape imminent shading from neighboring trees.

Most shade-avoidant species allocate more biomass to stems than to roots under low R/FR [17]. Examples of this response can be found in *Pinus sylvestris* L. [49] or silver birch [24], which showed an increase in the aboveground/belowground biomass ratio under low R/FR. In contrast, shade-tolerant species often show a reduction or no change in aboveground/belowground biomass ratio when exposed to low R/FR environments [11,50,51]. In our study, the aboveground/belowground biomass ratio was reduced in cvs. Arbequina and Coratina by low R/FR using the FR mirrors due to reduced total leaf weight per plant, but it was not reduced in cv. Arauco (Table 2). Again, these results are consistent with these two olive cultivars showing shade-tolerant characteristics. It has also been suggested that shade-avoiding responses are attenuated under water stress (as reviewed by [52]). Given that olive cultivars were selected for under mostly semi-arid, water-limited conditions, preferential allocation to aboveground biomass in response to impending shade would likely not have been beneficial if competition for soil water with neighboring plants was the most limiting factor for plant growth. From an agricultural perspective, the reductions in aboveground/belowground ratio would facilitate adaptation to SHD orchard systems by contributing to reduced tree crown size and by favoring root water absorption.
In addition to the FR mirrors, green vegetation fences (GF) were also used to horizontally reflect light to simulate the presence of neighboring plants. The GF had similar R/FR values compared with the FR mirrors, but the difference between the control fence and the GF in R/FR ratio was less than expected due to some reflection of FR light by the control fence (Figure 5). Leaf area per plant, internode length, and leaf biomass tended to be lower with the GF compared with the control fence in cvs. Arbequina and Coratina (Figure 7; Tables 3 and 4), but statistically significant differences were not detected (Figure 6; Tables 1 and 2). It is likely that the combined difference in the R/FR and B/G ratios between the GF treatment and the CF (Figure 5) was not large enough to cause significant morphological responses. In grape vines, morphological responses were observed when B/G was reduced to less than 0.04, but not with lesser B/G ratio reductions [53]. Additionally, morphological responses to FR reflected by green vegetation were considered to be proportional to the reduction in the R/FR ratio in silver birch [24]. A cluster analysis of the GF and FR mirror data did group the GF and FR mirror treatments together versus the two controls (CF and control mirror), which indicates some similarity in the responses to these two treatments (Figure 8).

5. Conclusions

Different spatial arrangements of potted olive trees showed that increasing LAI modifies the light quality environment by lowering horizontal R/FR and B/G ratios even before direct shading between neighboring trees occurs. Furthermore, reductions in horizontal R/FR using FR mirrors led to cultivar-specific responses in individual leaf area and aboveground/belowground biomass ratio during a controlled experiment. These first results in olive trees suggest that knowledge of cultivar differences in response to light quality may be relevant for understanding adaptation to SHD orchards and potentially for identifying cultivars to be used in SHD. However, further studies in more cultivars using different experimental approaches and measuring physiological variables, including photosynthesis and reproductive variables such as flowering, are needed to expand our limited knowledge.

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