



Responses of vegetative growth and fruit yield to winter and summer mechanical pruning in olive trees



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ABSTRACT

Mechanical pruning has become increasingly common in olive orchards, particularly under high tree densities. Large cutting disks make heading cuts at a single canopy depth without discriminating between branch thickness, size, or type of branch. The objectives of this study were to: (i) quantify the responses of vegetative growth over two growing seasons and yield components over three seasons following different intensities and moments of application of mechanical pruning; and (ii) evaluate some leaf morphology and gas-exchange characteristics of the remaining leaves after pruning. Five year-old olive trees with high crop load (cv. Arbequina) were pruned towards the end of the winter (W) or early summer (S). Three intensities of winter pruning representing different distances (0.25, 0.50, 0.75 m) from the outer canopy surface were applied, while there was only a single summer pruning treatment (0.75 m). The vegetative growth variables measured after pruning included new branch number and length, new leaf number, and increase in trunk cross sectional area. Reproductive variables included fruit and oil yield, fruit number, fruit weight, and oil content per fruit. Growth of new branches increased significantly with winter pruning intensity while delaying pruning to early summer reduced regrowth to the level of the unpruned control. Despite differences in yield in individual years between the unpruned control and the winter pruning treatments, the average yield over the three years after the winter pruning event was similar between all trees. Delaying the intense pruning to summer was associated with some reduction in yield, and moderate winter pruning (0.50 m) appeared to partially reduce alternate bearing. When measured shortly after winter pruning, specific leaf mass of the remaining leaves decreased steadily as the level of winter pruning increased, which is consistent with prior shading within the tree. The leaf net photosynthetic rate per unit mass was also different between pruning treatments. In conclusion, our results contribute to filling the gaps in knowledge related to important aspects of olive tree responses to the intensity and timing of mechanical pruning.

1. Introduction

There is a growing trend in the use of mechanical pruning in modern olive groves. The replacement of manual by mechanized pruning is in large part due to the increase in labor costs (Peça et al., 2002; Dias et al., 2012). Mechanical pruning in olive is performed by large cutting disk assemblies mounted onto a tractor or other vehicle. Discs make cuts at a single prescribed canopy depth and angle, which results in a uniform exterior canopy surface, without discriminating between branch thickness, size or type of branch. Such pruning alters the growth and development of individual trees and hedgerows because eliminating the branch apices leads to the reestablishment of hormone and nutrient relationships to the numerous remaining lateral buds on each branch (Génard et al., 1998). However, mechanical pruning can be an

advantageous management tool for maintaining an adequate canopy size for commercial harvesters, improving light distribution, and reducing alternate bearing (Connor et al., 2014).

Whether it be manual or mechanical pruning, olive tree pruning is most often conducted during the winter when there are few other management tasks to perform. Although this period does coincide with minimal shoot extension, little information is available for olive trees as to what this choice or the use of different training systems entail for subsequent branch growth and fruit yield (Aïachi Mezghani et al., 2012). Indeed, winter pruning in fruit trees has often been associated with excessive shoot growth (Mika, 1986; ; Sihan et al., 2005). As has been shown in apple, summer pruning may offer some benefits including improved fruit illumination, increased fruit size, reduced vegetative growth, and reduced canopy transpiration under high plant

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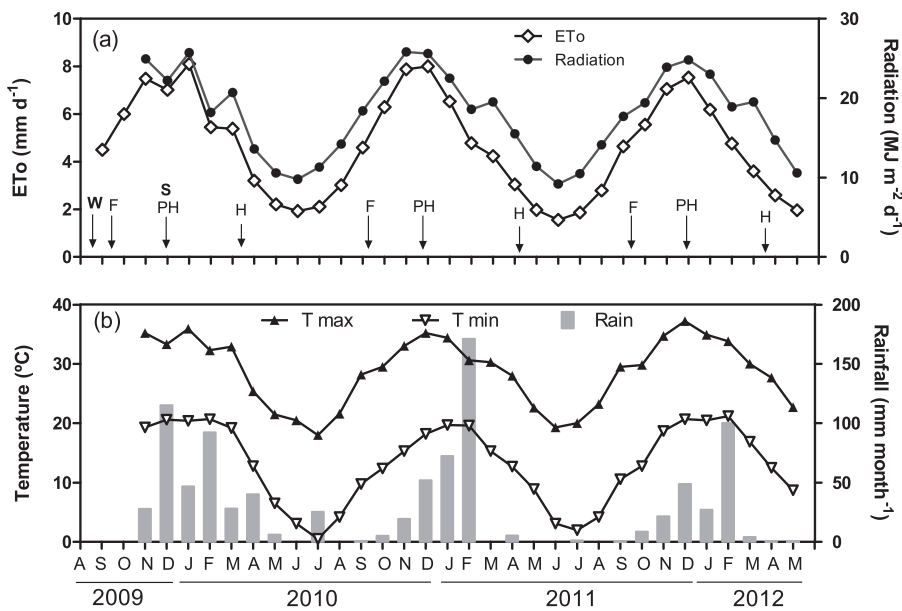


Fig. 1. Reference evapotranspiration (ETo) and solar radiation (a) as well as maximum (Tmax) and minimum (Tmin) temperature and rainfall (b) during the experiment (August 2009–May 2012). The ETo, solar radiation, and temperature values are average daily values for each month, while rainfall values are monthly totals (mm month⁻¹). The arrows indicate the dates of winter (W) and summer (S) pruning, flowering (F), pit hardening (PH), and harvest (H) for each year.

density (Mika, 1986; Forshey and Elfving, 1989; Li et al., 2003a,b). In olive, summer pruning is not a common practice, although eliminating the uppermost canopy growth (i.e., ‘topping’) from olive hedgerows during the summer before mechanical harvesting is increasingly applied.

In woody species, it is generally assumed that increasing the pruning intensity will result in more shoot growth following the pruning event. For example, Zeng (2003) observed that increasing the leaf area removed by pruning in *Ficus*, *Cinnamomum*, and *Pinus* favored biomass partitioning to leaves with pruned trees reaching leaf areas similar to those of unpruned trees one year after pruning. Additionally, new shoot elongation in peach increased with winter pruning intensity when pruning was conducted for three consecutive years (Siham et al., 2005). In olive, descriptive information suggests that post pruning vegetative growth responds strongly to pruning intensity (Gucci and Cantini, 2000), but quantification of the number and length of new shoots is needed over multiple growing seasons to design long-term pruning protocols in high density orchard systems.

The net carbon fixed by whole trees after pruning likely depends on factors such as the amount of leaf area removed, the photosynthesis of the remaining leaves, and canopy shape. In apple, the carbon fixed decreased proportionally with leaf area removed (13–64%) after summer pruning (Li et al., 2003a). Pruning of low branches in managed forest stands of *Eucalyptus* increased the net leaf CO₂ assimilation rate of the remaining branches after a winter pruning that was attributed to an increase in leaf conductance (g_l) (Pinkard et al., 1998; Pinkard, 2003; Medhurst et al., 2006). Using a modelling approach in olive, Fernández et al. (2008) have proposed that pruning olive trees from a spherical shape to truncated spheres (i.e., removing the top of the crown) may increase net carbon gain because it would increase the proportion of leaves exposed to sunlight. However, this would be affected by the photosynthetic characteristics of the remaining leaves under greater light levels, which are likely related to the canopy depth of the leaves prior to pruning (Larbi et al., 2015).

Studies focused on the quantitative responses of fruit tree species to mechanical pruning are scarce, although significant progress has been made recently in grapevines concerning the maintenance of training systems through mechanical pruning using specialized machinery (reviewed by Poni et al., 2016). In avocado and olive, studies of mechanical pruning are limited to yield comparisons between pruned trees and an unpruned or manually-pruned control (Morris and Cawthon, 1981; Giassetta and Zimbalatti, 1997; Thorp and Stowell, 2001; Poni et al., 2004; Dias et al., 2012). In this regard, there is no information

available in olive trees on the intensity or timing of mechanical pruning for maintaining canopy size without too adversely affecting yield and its components.

Thus, the objectives of the study were to: (i) quantify the responses of vegetative growth over two growing seasons and yield components over three seasons following different intensities and moments of application of mechanical pruning; and (ii) evaluate some leaf morphology and gas-exchange characteristics of the remaining leaves after pruning.

2. Materials and methods

2.1. Experimental site and pruning treatments

The experiment was conducted from August 2009 to April 2012 in a commercial olive orchard (*Olea europaea* cv. Arbequina) located 20 km north of the city of La Rioja, Argentina (lat. 29° 17' S, long. 66° 45' W; 444 m above sea level). The trees were 5 years-old at the beginning of the experiment with a north-south row orientation. The tree spacing was 6 m within rows and 8 m between rows (208 trees ha⁻¹). The soil was sandy loam in texture with a deep homogenous profile.

The orchard was within the Arid Chaco phytogeographic region and the climate is generally characterized by fairly mild, dry winters and very hot summers when torrential rainfall events often occur (Searles et al., 2011). The average daily reference evapotranspiration (ETo) values during the experimental period ranged from 1.6 mm d⁻¹ during the winter to 8.1 mm d⁻¹ during the summer months (Fig. 1a) for an annual ETo of about 1700 mm y⁻¹. The average maximum daily temperature ranged from 18.0 °C during the winter to 37.2 °C during the summer months with average minimum temperatures between 0.5 °C and 21.2 °C (Fig. 1b). Rainfall was about 340 mm y⁻¹ and was concentrated mainly in the summer months.

We employed fairly young, mid-sized trees for simulating mechanical pruning in this study because detailed measurements of very large, 5-m-tall hedgerows grown at low tree densities (200–400 trees ha⁻¹) are impractical for a large number of trees (Cherbiy-Hoffmann et al., 2012), and higher tree density hedgerow orchards were not yet available in our region. At the beginning of the study prior to pruning, the average canopy depth and diameter were 2.7 m and 2.2 m, respectively. Canopy depth was defined as the tree height minus the skirt-to-ground distance. Canopy diameter measurements were made every 0.50 m in height above ground level in the E-W and N-S directions to calculate the average canopy diameter. The initial canopy volume was estimated to

be about 7 m^3 before the pruning treatments were imposed, an estimate based the assumption that the tree crown was hemispherical in shape ($V = \frac{2}{3}\pi r^2 D$) (Del Río et al., 2005), with r being the canopy radius and D being the canopy depth.

Pruning was performed on individual trees in an “on” year with fruit load being high ($> 3400 \text{ fruit m}^{-3}$) for this cultivar according to Trentacoste et al. (2010). We conducted the experiment with “on” trees because a prior pruning study under similar climatic conditions with low yield, “off” trees (Cherbiy-Hoffman et al., 2012) indicated that branch growth after pruning was too excessive. The trees used in the current experiment had not been previously pruned except for removing suckers at the base of the tree. Neighboring trees were not pruned because of the free distance between tree canopies (3.7 m within the row; 5.8 m between rows). Three winter pruning (W) treatments and a single, early summer pruning (S) treatment were evaluated along with an unpruned control (CON). Winter pruning was done on August 24, 2009 when vegetative and reproductive buds could be easily distinguished, and summer pruning was conducted on December 1, 2009 just after massive pit hardening. The winter and summer pruning treatments were implemented using manual clippers by pruning both the entire east and west sides of the trees at different distances from the outer canopy surface. Pruning all branches at a given distance simulated the mechanical disk pruning technique that is increasingly common in many commercial olive orchards. The distances from the outer surface were 0.25 (25W), 0.50 (50W), and 0.75 m (75W) for the winter pruning treatments and 0.75 m (75S) for the summer pruning treatment. After pruning, the average tree diameter in the east-west direction was 1.70, 1.20, and 0.80 m for the three pruning distances. The tree canopy volumes were then recalculated by subtracting the pruned canopy volume from the original volume. The pruned canopy volume on each side of the tree canopy was estimated as a spherical sector ($V = \frac{1}{3}\pi p^2(3r - p)$), with r being the canopy radius and p the pruning distance from the outer canopy surface. The top of the trees was not pruned in any of the treatments during the experimental period because the height ($< 3.0 \text{ m}$) of the trees at the beginning of the experiment was much less than the 3.5 m maximum allowed for by many over-row harvesters.

The experimental design was a completely randomized block design with six blocks (Fig. 2). Each block consisted of a total of five trees including one tree each from all of the pruned treatments and the control. Thus, 30 trees were used during the study. Biomass removed by the different pruning treatments was weighed in the field using a

portable scale. Twelve branches per tree were taken to the laboratory in humidified plastic bags for measuring branch diameter at the point of pruning using an electronic caliper and for determining separately the fresh and dry weights of the stems, leaves, and fruit of each branch. The vegetative material was dried at $75 \text{ }^\circ\text{C}$ in a forced-air oven until a constant weight was reached. Leaf area per branch was estimated from leaf disk weight taken with a hole punch and total leaf weight.

All trees were irrigated to meet 100% of their crop evapotranspiration requirements using a crop coefficient (Kc) of 0.7 during the growing season and a Kc of 0.4 during the winter months (Rousseaux et al., 2009). Irrigation levels were adjusted to the canopy size of each pruned treatment and the control by calculating a reduction coefficient (Kr) that represented the ground area shaded by the tree canopy (Feres et al., 1981). The Kr value of the control was 0.30 the first year of the experiment and 0.40 the second year. The Kr values of the pruned treatments (25W, 50W, 75W, 75S) were 0.27, 0.22, 0.19 and 0.19 during the first year, respectively. These values increased to 0.37, 0.31, 0.33, and 0.29 the second year. The required amount of irrigation per treatment was obtained by employing different combinations of four drip emitters per tree with different drip rates ($2\text{--}4 \text{ l h}^{-1}$). The conductivity of the irrigation water was less than 2 dS/m .

2.2. Trunk and branch growth

Trunk growth measurements were made during the course of the growing season in which the pruning treatments were implemented (2009–10; Year 1) and during the following season (2010–11; Year 2). The trunk circumference was determined every 45 d at a trunk height of 30 cm using a flexible measuring tape. The cumulative increase in trunk cross-sectional area (TCSA) each season was calculated from these measurements using the equation $TCSA = \pi r^2$, where r is the trunk radius.

Twelve base branches (BB) per pruned tree were selected to quantify vegetative growth. The term ‘base branch’ refers to the underlying woody support structure just below the point of pruning. Six BB were marked on each of the two pruned sides per tree by selecting three branches at two heights (1 and 2 m above the ground). In the control trees, 12 well illuminated branches were selected in the outer portion of the canopy whose diameters at their base were similar to those of the 25W base branches. The number of new branches, branch length, and leaf numbers associated with the BB and marked control branches were determined in the first and second growing seasons. The measurement dates coincided with the end of winter rest (August), beginning of active oil accumulation (November), mid-summer (February), and post-harvest when vegetative growth was minimal (April). For the 75S treatment, the first measurements were made in February 2010.

2.3. Yield components

At harvest, fresh fruit yield per tree, number of fruit per tree, individual fruit dry weight, oil yield per tree, and fruit oil content were determined. The trees were harvested manually on March 26 (Year 1), April 14 (Year 2), and March 28 (Year 3) when visual observation indicated that the fruit were at the veraison stage of maturity (i.e., the skin of the fruit was reddish in color). Veraison was used to approximate the date of the fruit reaching maximum oil concentration (Beltrán et al., 2004). From the fresh fruit yield of each individual tree (kg tree^{-1}), a sample of 2 kg was taken to the laboratory in a cooler where the fresh weight of a sub-sample of 100 fruit was determined. The fruit were then dried in an oven for 6 d at $70 \text{ }^\circ\text{C}$, and individual fruit weight was calculated on a fresh and a dry weight basis. The total number of fruit per tree was estimated by dividing the total fruit fresh weight per tree by the average individual fresh fruit weight. Fruit maturity index (MI) was determined on another sub-sample of 100 fruit by classifying the fruit from 0 to 7 according to skin color and pulp. The MI was roughly consistent with the visual observation of the fruit being at

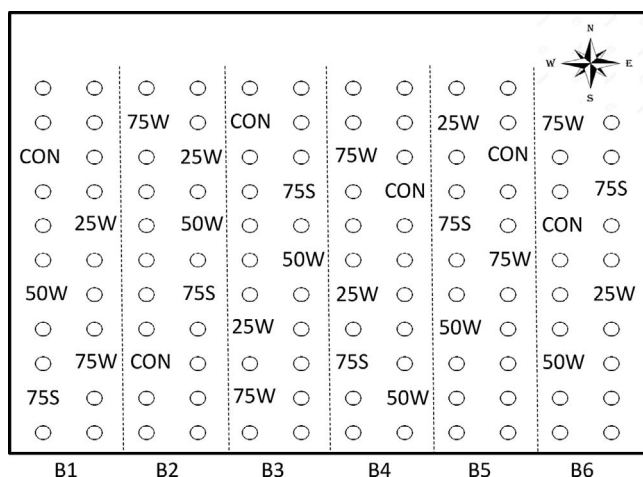


Fig. 2. Diagram of the experimental design for the winter (25W, 50W, and 75W) and summer (75S) pruning treatments and the unpruned control (CON). Individual trees were used as experimental plots in a completely randomized block design with 6 blocks (B1 to B6). The numbers 25, 50, and 75 in the treatment abbreviations indicate the width (cm) of the layer removed by pruning from the outer canopy surface. The tree spacing was 6 m within rows and 8 m between N-S oriented rows.

veraison (i.e., MI of 2 or 3) with the average MI being 2.5, 3.7, and 3.7 for Years 1, 2, and 3; respectively. No differences in MI were apparent between the pruning treatments and the control. The rest of the sampled fruit (approx. 1 kg) were ground in a hammer mill. The oil concentration (%) of the dry paste was determined using magnetic resonance equipment (SLK AC-100, Spinlock SRL, Cordoba, Argentina) in Years 1 and 2, and by hexane extraction for 6 h with a Soxhlet apparatus in Year 3. Fruit oil content was calculated as g oil fruit⁻¹ based on individual fruit dry weight and fruit oil concentration (%). Oil yield per tree (kg tree⁻¹) was a function of fruit yield per tree and oil concentration.

2.4. Maximum leaf photosynthesis and conductance after winter pruning

The maximum net leaf photosynthetic rate (*A*) was measured on two fully expanded leaves per tree positioned at the outer canopy surface one month after winter pruning (September 24, 2009) using a portable gas exchange system (CID Inc., model CI-310, Vancouver, WA, USA). The photosynthetic photon flux density (PPFD) in the leaf chamber was 1300–1600 μmol m⁻² s⁻¹ using natural lighting at the time of the measurements (10–12 solar time) on east-facing leaves. Air flow into the leaf chamber was 0.4 l min⁻¹ and air temperature was never more than 3 °C above ambient temperature due to a Peltier cooling system at the base of the leaf chamber. The measured leaves were collected and transported to the laboratory in airtight bags for the determination of leaf area and specific leaf mass (SLM). Leaf conductance (gl) was concurrently determined on similar leaves using a diffusion porometer (Delta-T Devices Ltd, model AP4, Cambridge, UK).

2.5. Statistical analyses

Branch growth, TCSA, yield and its components were analyzed with general linear models of ANOVA for repeated measures in time because the measurements were performed on the same experimental units (i.e., trees) over multiple dates and years. Differences between means ($P < 0.05$) were then evaluated using LSD tests. TCSA, total number of new branches, total length of new branches, and total number of leaves on the new branches were transformed to meet the normality and homogeneity of variance assumptions using appropriate transformations including $\ln(y + 1)$, \sqrt{y} , and arc cosine. The above analyses were all conducted using InfoStat software (version 2014, Universidad Nacional de Córdoba, Argentina). Simple linear regressions were fitted to the relationships between branch growth or yield and its components and the leaf area removed in the winter and summer pruning events using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). Lastly, multivariate principal component analysis was used to examine relationships at the end of the growing season between vegetative and yield variables with pruning treatment and the moment (winter or summer) when pruning was performed.

3. Results

3.1. Biomass removed by pruning treatments

The dry weight of branches and leaves removed with pruning significantly increased with the winter pruning intensity (Fig. 3). In contrast, no differences between the severe winter (75W) and summer (75S) pruning treatments were observed when only branch and leaf weight were considered. Inclusion of the fruit for the 75S treatment led to an additional 1.7 ± 0.14 kg being removed per tree on a dry weight basis. The biomass removed by pruning accounted for 6, 21, and 40% of the crown volume for the 25W, 50W, and the 75W-75S treatments.

3.2. Trunk and branch growth

The TCSA increased in all treatments during the growing season in

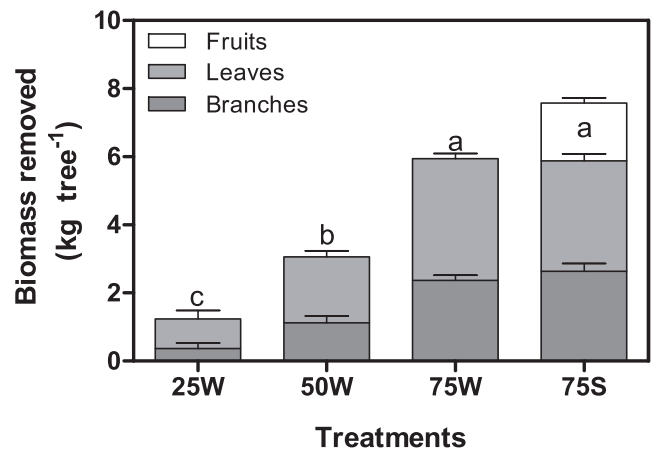


Fig. 3. Dry weight of the branches, leaves, and fruit removed by the winter (25W, 50W, and 75W) and summer (75S) pruning treatments. The numbers 25, 50, and 75 in the treatment abbreviations indicate the width (cm) of the layer removed by pruning from the outer canopy surface. Fruit were only present in the summer pruned material. Each bar represents the average \pm one standard error ($n = 6$ trees per treatment). The letters above the bars indicate significant differences between treatments ($P < 0.05$) for the sum of branches + leaves.

Years 1 and 2 (Fig. 4). On average, TCSA increased 12.8 cm² during Year 1, although no significant differences were apparent between the control and the pruning treatments (Fig. 4a). In Year 2, TCSA increased 22.4 cm² in the control, which approximately doubled the TCSA increase of the various pruning treatments ($P < 0.05$, Fig. 4b).

The number of new branches per BB as well as their total length and total number of leaves increased with pruning intensity in both Years 1 and 2 (Fig. 5). As the growing season progressed, differences between the more strongly pruned winter treatments (50W, 75W) became evident for all of the measured variables compared to the lightly pruned winter treatment (25W), the summer pruning treatment (75S), and the control. Growth was higher in Year 2 than in Year 1 with the total length of new branches in the 50W and 75W pruning treatments reaching near 150 cm per BB in Year 2 and about 100 cm per BB in Year 1 (Fig. 5c, d).

3.3. Yield and its components

Except for a decrease in fruit number per tree in the early summer pruning treatment (75S), the three-year averages of yield and its components were not significantly affected by the pruning treatments (Table 1). However, there were significant interactions for yield and all of its components between pruning and year (Table 1; Fig. 6). For example, the pattern of fruit yield in the control trees showed high production in Year 1, low yield in Year 2, and fairly high yield in Year 3 (Fig. 6a). Both the severely pruned winter (W75) and summer treatments (S75), which had 40% of the tree crown volume removed the first year, showed an inverse yield pattern to that of the control. Oil yield showed a similar, but less pronounced pattern (Fig. 6b).

A pruning \times year interaction was also apparent for individual fruit dry weight and oil content. Both variables were significantly higher in the 75W and 75S pruning treatments in Year 1 than the control (Fig. 6d, e) possibly due to the tendency of fruit number to be lower in those trees (Fig. 6c). However, fruit weight and oil content were significantly lower in the severely pruned treatments than the control in Year 2. In Year 3, there was again a tendency for fruit weight and oil content to be higher in the W75 and S75 trees. Oil concentration (% of fruit dry weight) did not show any difference between pruning treatments including the control for any of the three years (data not shown).

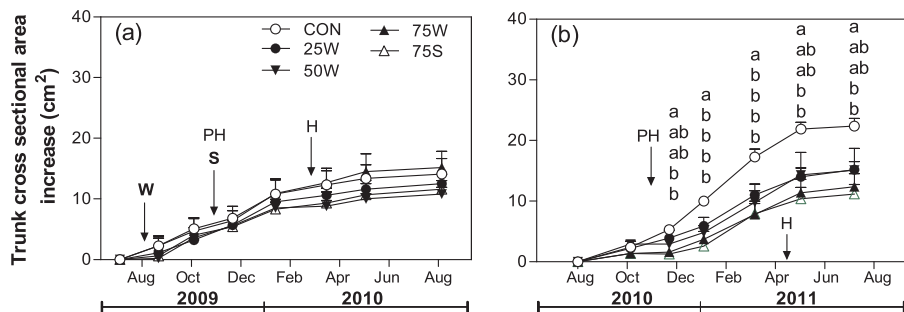


Fig. 4. Seasonal patterns of trunk cross-sectional area (TCSA) increase in Year 1 when winter (W) and summer (S) pruning were performed (a) and Year 2 when there was no pruning (b). The pruning treatments were 25W, 50W, 75W, and 75S with the numbers in the treatment abbreviations indicating the width (cm) of the layer removed by pruning from the outer canopy surface. Arrows indicate the dates of winter pruning (W), summer pruning (S), pit hardening (PH), and harvest (H). Each point represents an average \pm one standard error ($n = 6$ trees per treatment). The letters above the points for each measurement date indicate significant differences between treatments ($P < 0.05$).

3.4. Relationships between leaf area removed by pruning and subsequent branch growth and yield

To assess the potential of predicting vegetative growth and yield in the year that pruning was conducted, linear regressions were used. Linear regressions from Year 1 showed that the number of new branches per BB as well as their total length and leaf number increased significantly with the amount of leaf area removed (LAR) by winter pruning (Table 2). For each 1.0 m² of LAR, total branch length increased 3.4 cm and leaf number increased by 4.6. The vegetative growth of the summer pruning (75S) treatment did not respond strongly to pruning and was thus not included in the branch growth regression equations.

In contrast to branch growth, fruit and oil yield and fruit number per tree decreased significantly in Year 1 with the amount of LAR by pruning when including both the winter and summer pruning treatments. For each 1.0 m² of LAR, fruit yield decreased 0.45 kg tree⁻¹ and

fruit number decreased 458 fruit tree⁻¹. Individual fruit dry weight and oil content increased significantly with increasing LAR as might be expected due to partial compensation for the lower fruit number.

3.5. Principal component analysis

The first two principal components accounted for 43% (PC1) and 33% (PC2) of the total variance for the vegetative growth and yield variables analyzed for Years 1 and 2 (Fig. 7). The variables that had high, positive correlation coefficients for PC1 were fruit oil content and fruit dry weight as well as the three variables associated with new branches per BB (total number of branches, length, and number of leaves) (Table 3). In contrast, fruit number per tree had a negative coefficient for PC1. Given the weight of the variables and the trajectories of the vectors, treatments such as 50W and 75W that showed high new branch growth (especially in Year 2) also had larger fruit with higher oil content, but fewer fruit per tree. The variables that had high,

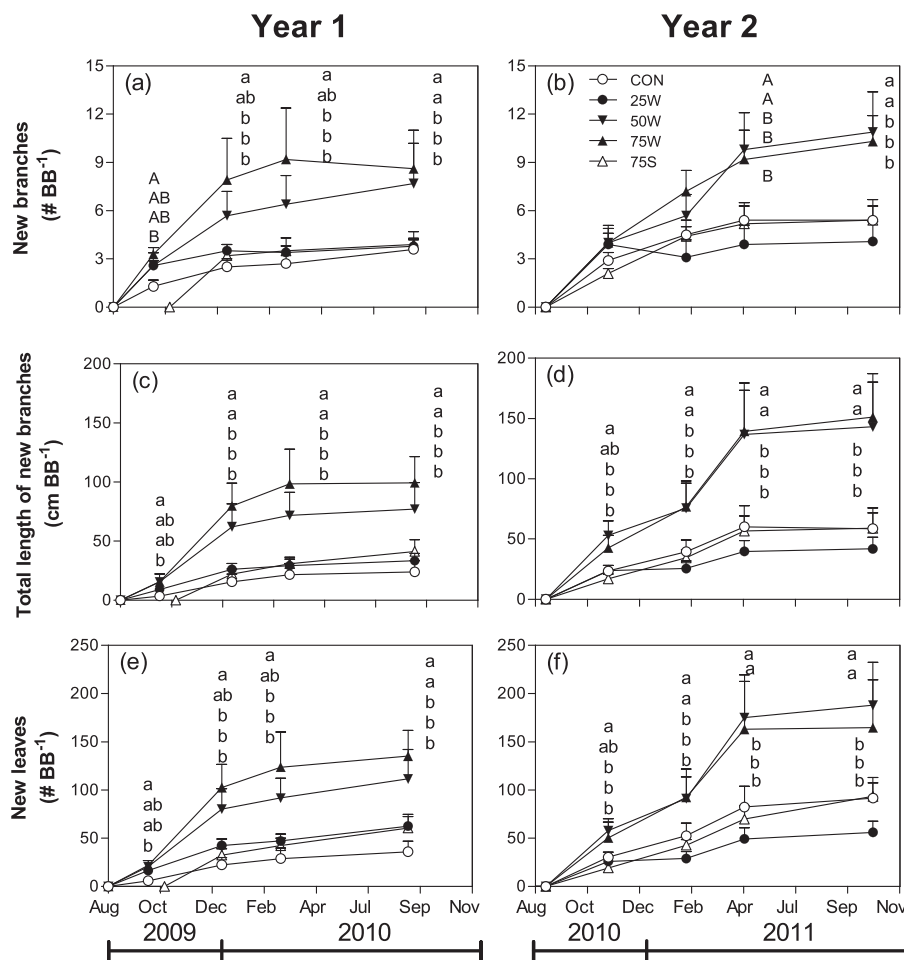


Fig. 5. Total number of new branches (a, b), total length of new branches (c, d), and total number of new leaves (e, f) per base branch (BB) in Year 1 when winter (W) and summer (S) pruning were performed (left panels) and Year 2 when there was no pruning (right panels). The pruning treatments were 25W, 50W, 75W, and 75S with the numbers in the treatment abbreviations indicating the width (cm) of the layer removed by pruning from the outer canopy surface. Each point represents an average \pm one standard error ($n = 6$ trees per treatment). The letters above the points for each measurement date indicate significant differences between treatments (lower case letters for $P < 0.05$; capital letters for $P < 0.1$).

Table 1

Analysis of yield and its components to pruning and year using repeated measures in time. The three-year averages of yield and its components are shown \pm one standard error for each pruning treatment and the control in the upper part of the table. The average for each year \pm one standard error is shown below. Different letters within a column indicate significant differences ($P < 0.05$) between pruning treatments or year.

Factors	Fruit yield	Oil yield	Fruit number	Fruit dry weight	Fruit oil content
	(kg tree ⁻¹)	(kg tree ⁻¹)	(# tree ⁻¹)	(g fruit ⁻¹)	(g fruit ⁻¹)
Pruning					
CON	20.31 \pm 2.5 a	2.51 \pm 0.30 a	12,461 \pm 2043 a	0.75 \pm 0.05 a	0.26 \pm 0.03 a
25W	20.04 \pm 2.6 a	2.84 \pm 0.42 a	12,836 \pm 2258 a	0.76 \pm 0.05 a	0.26 \pm 0.02 a
50W	19.35 \pm 1.8 a	2.65 \pm 0.32 a	12,408 \pm 1837 a	0.74 \pm 0.05 a	0.26 \pm 0.02 a
75W	22.72 \pm 2.6 a	3.13 \pm 0.36 a	13,520 \pm 1760 a	0.71 \pm 0.03 a	0.26 \pm 0.02 a
75S	15.42 \pm 1.7 a	2.15 \pm 0.31a	8546 \pm 1134 b	0.76 \pm 0.04 a	0.27 \pm 0.02 a
Year					
1	20.26 \pm 0.9 a	2.83 \pm 0.19 a	18,673 \pm 1187 a	0.54 \pm 0.01 b	0.16 \pm 0.00 c
2	21.54 \pm 1.8 a	3.27 \pm 0.29 a	9897 \pm 1101 b	0.85 \pm 0.02 a	0.36 \pm 0.01 a
3	16.91 \pm 2.2 a	1.87 \pm 0.24 b	7293 \pm 1011 c	0.86 \pm 0.02 a	0.27 \pm 0.01 b
P value					
Pruning (P)	0.32	0.36	0.03	0.34	0.35
Year (Y)	0.17	< 0.001	< 0.001	< 0.001	< 0.005
P x Y	0.006	0.02	0.006	0.002	0.002

positive correlation coefficients for the PC2 were fruit and oil yield and fruit number, while the increase in TCSA showed a negative coefficient. This indicates that yield and fruit number were positively related and that both these variables were inversely related to TCSA. In Year 2, the variability associated with the control was strongly related to TCSA, and yield in the control trees was low.

3.6. Specific leaf mass and gas-exchange variables after winter pruning

Consistent with prior shading within the tree, specific leaf mass at the post-pruned outer surface of the canopy decreased steadily as winter pruning intensity increased when measured one month after pruning (Table 4). The leaf net photosynthetic rate (A) per unit mass increased with pruning, and leaves of the 50W and 75W pruning treatments had higher values of A than the unpruned control leaves. No significant differences were observed when A was expressed per unit of leaf area. The leaves of the 50W and 75W pruning treatments also showed higher transpiration (E) per unit mass and some tendency for differences per unit of leaf area. Similarly, leaf conductance (g_l) was higher in leaves of 75W than in control leaves (Table 4).

4. Discussion

Mechanical pruning of olive trees, or combining mechanical pruning with manual interventions, has become more common in recent years due to rising labor costs and increasing tree density (Ruis and Lacarte, 2010; Connor et al., 2014; Vivaldi et al., 2015). Our study addressed the impact of mechanical pruning on vegetative regrowth over two years, and yield over three years. With the aim of reducing post-pruning vegetative growth, pruning was performed in a high fruit load year in a cultivar (Arbequina) considered to have low vigor in its place of origin in the Mediterranean Basin (IOOC, 2000; Rosati et al., 2013).

Despite fairly high fruit loads, the moderate (50W) and severe (75W) winter pruning treatments were associated with high levels of new branches and total branch length the first year under our warm climate conditions in northwestern Argentina (Fig. 5). Significant increases in vegetative growth in response to pruning also may occur in other fruit tree species even under more temperate conditions (Génard et al., 1998; Stephan et al., 2007; Bussi et al., 2011; Pasa and Einhorn, 2014). In Year 2, in which no pruning interventions were carried out, branch growth remained high in these pruned trees with more than 150 cm of new branch being produced per base branch. This response may have occurred due to changes in carbon partitioning in response to

pruning or long-term changes in hormone levels related to the removal of branch apices (i.e., the heading cuts) with mechanical pruning (Mika 1986; Génard et al., 2008).

Alternate bearing often occurs in olive trees with a year of high fruit load (“on” year) being followed by a year of low fruit load (“off” year) (Morettini, 1972; Monselise and Goldschmidt, 1982; Lavee, 2007). Year 1 was an “on” year in the unpruned, control trees with high fruit number and yield (Fig. 6), while their values decreased somewhat with winter pruning intensity as would be expected by eliminating fructification sites through non-selective pruning (e.g., Kumar et al., 2010). In Years 2 and 3, fruit number and yield in unpruned trees were influenced by the previous year fruit load with some tradeoff between fruit number and fruit weight and oil content (Fig. 6). There are indications that the vegetative growth of the unpruned, control trees was also regulated by the fruit load as has been seen in previous studies (e.g., Dag et al., 2010; Fernández et al., 2015). Both trunk and branch growth (Figs. 4, 5) were lower in the unpruned trees in the high fruit load year (Year 1) than in the low fruit load year (Year 2) as would be expected due to photo-assimilate limitations. However, the moderately (50W) and severely (75W) pruned trees showed greater branch growth the second year despite yield being greater than the first year. Interestingly, the high branch growth in the pruned trees in Year 2 did not lead to an increase in fruit number in Year 3. This result could be related to high levels of gibberellin inhibiting floral induction in these juvenile, rapidly growing branches (reviewed by Bangerth 2009), and shows that yield is difficult to predict after mechanical pruning if based solely on vegetative growth.

Despite the differences in yield in individual years between the control and the winter pruning treatments, the average yield over the three years after the pruning event was similar between all trees (Table 1). Using a combination of annual mechanical and manual pruning, Vivaldi et al. (2015) also did not report reductions in yield over three years for ‘Arbequina’ cultivated in a super high density, hedgerow orchard. Our study and that of Vivaldi et al. (2015) employed trees of similar age (5 or 6 years-old at the beginning of the experiment) and initial canopy width (approx. 2 m). Although yield was always fairly high in our study, yield fluctuations were lower in 50W than in the rest of the pruning treatments and the unpruned control (Figs. 6, 7). Thus, removing the outermost 50 cm of the hedge in an “on” year could be used to both control tree size and to partially reduce alternate bearing. The removal of 50 cm of canopy at the beginning of the experiment was equivalent to 3 kg of pruned biomass per tree (Fig. 3). In Vivaldi et al. (2015), an amount of 2 kg per tree were mechanically pruned in the first and third years with almost no mechanical pruning

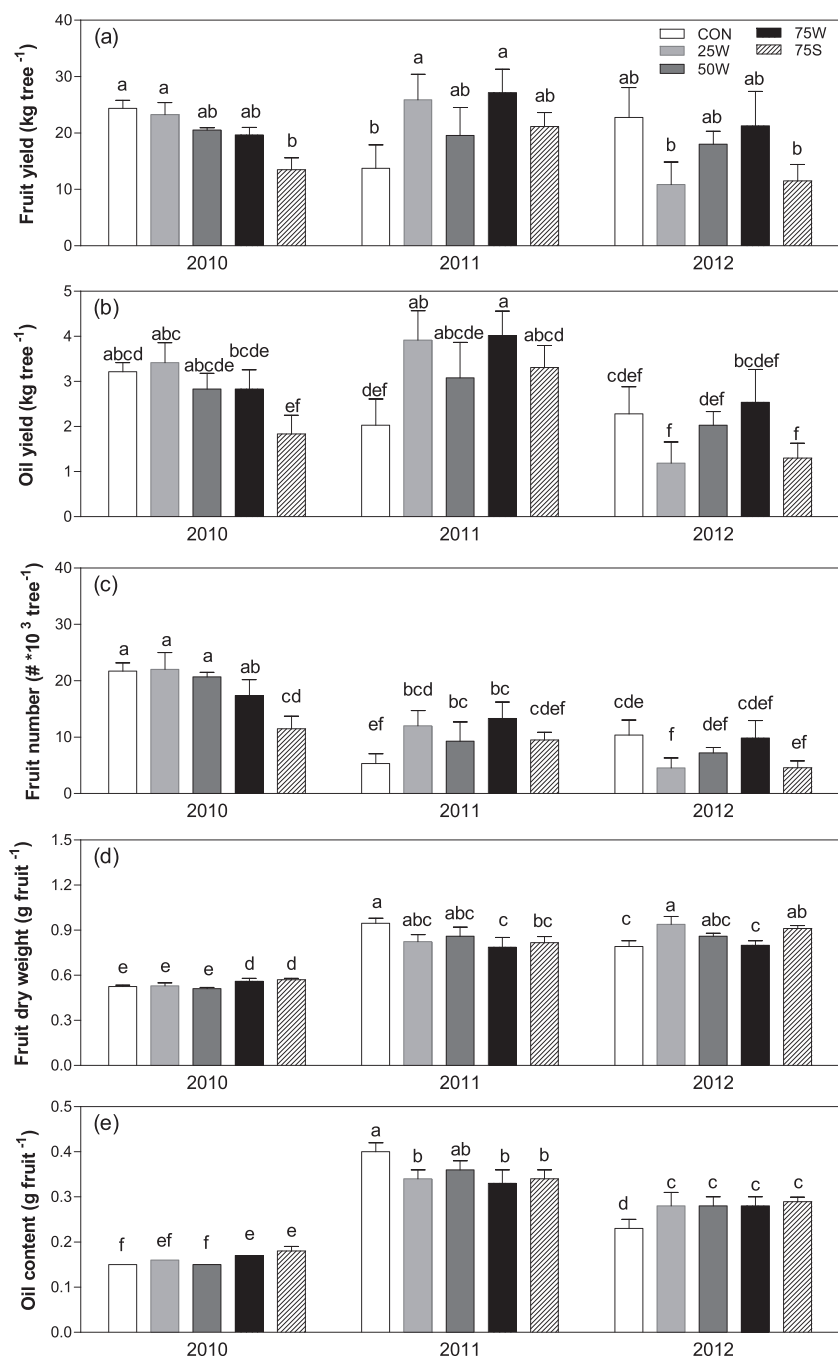


Fig. 6. Fresh fruit yield (a), oil yield (b), fruit number (c), individual fruit dry weight (d), and oil content (e) measured in Year 1 when winter (W) and summer (S) pruning were performed and Years 2 and 3 when there was no pruning. The pruning treatments were 25W, 50W, 75W, and 75S with the numbers in the treatment abbreviations indicating the width (cm) of the layer removed by pruning from the outer canopy surface. Each bar represents an average ± one standard error (n = 6 trees per treatment). The different letters located above the bars indicate significant differences between treatments in a given year and among years (P < 0.05).

Table 2

Linear regressions between branch growth, yield, and its components during Year 1 and leaf area removed by pruning (LAR, m²). Vegetative growth regressions do not include the summer pruning treatment (75S), while regressions for yield and its components include all pruning treatments. All regression coefficients are significant (P < 0.05).

Variable	Model	R ²
Number of new branches (# BB ⁻¹)	y = 3.5 + 0.19 (LAR)	0.31
Length of new branches (cm BB ⁻¹)	y = 25 + 3.4 (LAR)	0.57
Number of new leaves (# BB ⁻¹)	y = 42.5 + 4.6 (LAR)	0.55
Fruit yield (kg tree ⁻¹)	y = 24.2 - 0.45 (LAR)	0.35
Oil yield (kg tree ⁻¹)	y = 3.32 - 0.06 (LAR)	0.17
Fruit number (# tree ⁻¹)	y = 22620 - 458 (LAR)	0.25
Fruit dry weight (g fruit ⁻¹)	y = 0.52 + 0.0032 (LAR)	0.29
Fruit oil content (g oil fruit ⁻¹)	y = 0.15 + 0.0012 (LAR)	0.37

the second year.

The timing of pruning strongly influenced vegetative growth. By delaying pruning until the beginning of the summer (75S), the new branch growth was similar to that of the control and much lower than the growth observed with same pruning intensity when conducted towards the end of the winter (75W; Fig. 5). Similar to what has been reported for grapevine (Friend and Trought, 2007), the summer pruning delayed bud break and allowed less time for new branch formation and growth. This suggests that summer pruning would be an appropriate time for removing canopy height that is in excess of mechanical harvester dimensions in order to avoid vigorous regrowth that is often unproductive (Cherbiy-Hoffmann et al., 2012).

In contrast, lateral hedge pruning may not be beneficial in the summer due to potential yield reductions. Fruit number per tree in Year 1 was reduced by 45% and 34% compared to the control and 75W pruning treatment; respectively, and fruit number over the three years

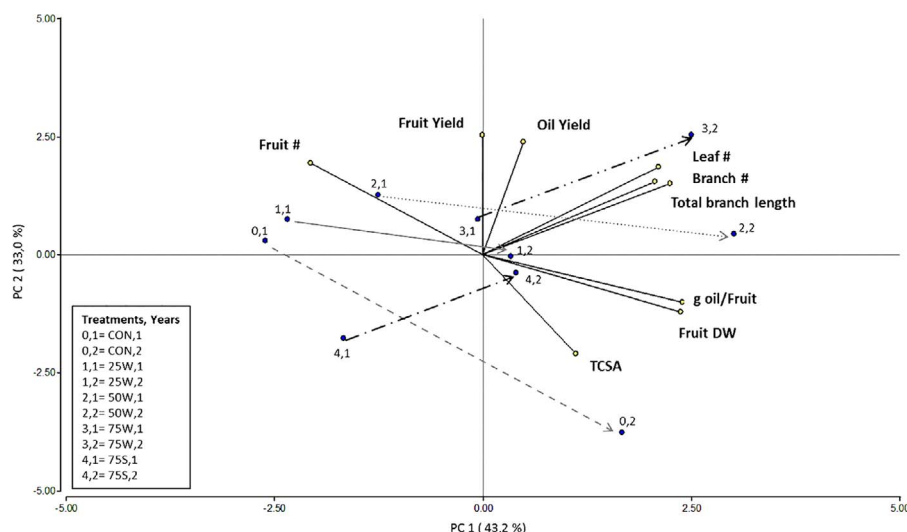


Fig. 7. Biplot of the effects of the pruning treatments and year using principal component analysis for Years 1 and 2. The analysis did not include data from Year 3 because vegetative growth was measured only in Years 1 and 2. Each point represents the average for a combination of pruning treatment and year (n = 6 trees per treatment). Codes for the combinations of pruning treatment and year are shown in the bottom left quadrant. The arrows indicate the trajectories between years in the biplot space for each pruning treatment.

Table 3
Correlation coefficients of the principal components (PC1 and PC2) for the different vegetative growth and yield variables. Correlation coefficients in bold were considered in the interpretation of the main components.

Variable	PC1	PC2
Fruit yield (kg tree ⁻¹)	-0.00078	0.79
Oil yield (kg tree ⁻¹)	0.17	0.74
Fruit number (# tree ⁻¹)	-0.73	0.6
Fruit dry weight (g fruit ⁻¹)	0.84	-0.38
Fruit oil content (g oil fruit ⁻¹)	0.85	-0.32
Number of new branches (# BB ⁻¹)	0.73	0.48
Length of new branches (cm BB ⁻¹)	0.80	0.47
Number of new leaves (# BB ⁻¹)	0.75	0.58
Increase in trunk cross sectional area (cm ²)	0.39	-0.65

was significantly less in 75S than in the control and the winter pruning treatments ($P < 0.03$, Table 1). Although there was not a statistically significant difference in yield over the three years (Table 1), the reduction in fruit number contributed to yield showing a tendency to decrease (-25%) under summer pruning. The lower fruit number in the 75S pruning treatment relative to 75W in Year 1 appeared to be related to greater fruit set on the unpruned north and south sides of the 75W trees (Albarracín, unpublished data). This could be due to an enhancement in the floral quality after the winter pruning as has been observed in mango (Sharma and Singh, 2006). Differential fruit set on pruned and unpruned sides of the tree would provide a concrete basis for alternating pruning between sides of olive hedgerows (Ruis and Lacarte, 2010; Lodolini et al., 2011).

In deciduous fruit trees, structural-functional models for predicting the response to pruning or fruit thinning have been developed (e.g.,

Table 4
Specific leaf mass (SLM), net photosynthesis (A), transpiration (E), leaf conductance (g_l), and water use efficiency (WUE) measured one month after the winter pruning in Year 1. Each value represents the average ± one standard error (n = 4 trees per treatment). Different letters within a row indicate significant differences between treatments (lower case letters for $P < 0.05$; capital letters for $P < 0.1$).

Variables	Pruning treatments			
	CON	25W	50W	75W
SLM (g m ⁻²)	293 ± 12 a	261 ± 16 b	223 ± 13 c	193 ± 7 d
A (μmol CO ₂ g ⁻¹ s ⁻¹)	0.10 ± 0.01 b	0.13 ± 0.02 ab	0.15 ± 0.01 a	0.17 ± 0.01 a
A (μmol CO ₂ m ⁻² s ⁻¹)	27.9 ± 2.7	31.8 ± 2.9	33.2 ± 0.9	32.4 ± 1.8
E (mmol g ⁻¹ s ⁻¹)	0.013 ± 0.02 c	0.019 ± 0.04 bc	0.023 ± 0.04 ab	0.026 ± 0.02 a
E (mmol m ⁻² s ⁻¹)	3.71 ± 0.52 B	4.68 ± 0.59 AB	4.90 ± 0.71 AB	4.95 ± 0.28 A
g _l (mmol m ⁻² s ⁻¹)	228 ± 30 b	256 ± 43 ab	254 ± 12 ab	278 ± 36 a
WUE	7.96 ± 1.1	6.94 ± 0.3	7.34 ± 1.2	6.68 ± 0.6

Balandier et al., 2000 for walnut; Lopez et al., 2010 for peach; Stephan et al., 2008 for apple). Such models are useful in fruit tree species with relatively few large fruit where pruning and thinning are quite often done manually and selectively. This approach is likely to be of limited use in olive because of the very large number of small fruit and because mechanical pruning removes branches non-selectively. The use of simple linear models showed that the increase in post-pruning branch growth was positively associated with leaf area removed (LAR) by pruning, explaining up to 57% of the variation in the data (Table 2). Additionally, fruit number and yield were negatively associated with LAR. Over a narrower range of mechanical pruning intensity, Vivaldi et al. (2015) did not observe a significant correlation between yield and biomass removed by mechanical pruning in fairly low vigor cultivars such as ‘Arbequina, but yield did decrease with biomass removed in more vigorously growing cultivars.

The principal component analysis biplot allowed us to better identify relationships between the studied variables (Fig. 7). The analysis separated branch growth, fruit size, and oil content on the first principal component (PC1) axis from variables such as yield and fruit number on the second principal component (PC2) axis. This confirms that high levels of post-pruning branch growth were negatively related to yield and fruit number (Figs. 5, 6; Table 2). A recent factorial study of deficit irrigation and fertilization in a super high density “Arbequina” orchard also identified a negative relationship between vegetative growth and yield when pruning was conducted manually every season using principal component analysis (Rufat et al., 2014).

The morphological and physiological characteristics of the leaves exposed to full sunlight after mechanical pruning has been little considered. The specific leaf mass (SLM) of the leaves located at the exterior of the canopy shortly after winter pruning was consistent with the

anticipated response of leaves grown in the shade (Table 4). The SLM was 11, 24 and 34% lower in the 25W, 50W, and 75W pruning treatments, respectively, when compared with leaves from the unpruned control. This response is similar to that observed in olive trees shaded under neutral density shade cloth (Gregoriou et al., 2007). A simulation model by Fernández et al. (2008) has suggested that the net carbon gain of olive trees would be increased when trees were manually pruned due to an increase in the proportion of sun-lit leaves. However, the photosynthetic characteristics of previously shaded leaves should be considered. In addition to the differences in SLM after pruning in our study, the values of photosynthesis and transpiration per unit weight were higher in leaves from pruned trees compared with the control. Although our data are limited in their temporal post-pruning coverage, they indicate the need to assess differences in the gas-exchange of previously shaded and new leaves in response to mechanical pruning.

5. Conclusion

Our results contribute to filling the gaps in knowledge related to important aspects of olive tree responses to the intensity and timing of mechanical pruning. When winter pruning was conducted in a year with high yield, the three-year average yield following the single pruning event was not affected by the different intensities of winter pruning. However, a moderate pruning of 50 cm from the edge of the crown for a standard 2 m-wide tree canopy decreased yearly fluctuations in yield under our experimental conditions. Although branch growth responded strongly to winter pruning for at least two growing seasons, principal components analysis suggests that branch growth was negatively correlated to yield and suggests that the potential consequences of vigorous regrowth must be carefully considered for specific tree densities and climate conditions. Finally, severe lateral pruning in the summer was associated with much lower vegetative growth, but summer fruit removal had a negative impact on the three-year average of fruit number with a 25% reduction in yield. Further studies are needed in more mature orchards at higher tree densities to better our understanding of orchard responses to pruning.

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