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Olive floral development in different hedgerow positions and orientations as affected by irradiance

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ABSTRACT

Irradiance received within the olive hedgerow canopy varies with respect to row orientation, spacing and hedge dimensions. These orchard management criteria offer the opportunity for improving productivity based on understanding the responses of yield-determining processes to irradiance. How irradiance influences inflorescence and flower development, the initial steps in fruit formation, are fundamental components of these processes. In this study we evaluated flowering and fruiting parameters in 5 hedgerow positions (defined by hedgerow side and vertical layer above soil) for N–S (North-South) and E–W (East–West) olive hedgerows (cv. Arbequina). The canopy layers and orientations provided a wide gradient of irradiance received and the relationship of estimated mean daily irradiance for annual and for short periods during floral development and initial fruit set was explored. The numbers of inflorescences and fruits per layer increased from the less illuminated base to more illuminated upper canopy layers. Axillary bud number per shoot also increased toward more illuminated positions, while the proportion of floral buds was unresponsive to the irradiance microenvironment at different positions within the hedgerows. Inflorescence length, node and flower number per inflorescence, and perfect flower percentage increased with position illumination. Ovary quality, indicated by ovule differentiation, was consistently high, independent of position, but ovary size showed some slight significant increases with illumination, mainly in the endocarp. Flowers/inflorescence, fruits/fruiting inflorescence and inflorescence and fruit number per position correlated positively and significantly with estimated irradiance similarly for annual and short periods (r range from 0.49 to 0.86). Despite improved flowering parameters with greater irradiance, no consistent differences among positions were found for percentage of inflorescences bearing fruit and fruit number per inflorescence. Instead, our results indicated that different fruit numbers among canopy positions were primarily due to an irradiance effect on vegetative growth, causing more and longer fruiting shoots and therefore more total flowering sites (nodes) per layer, with only a small contribution by inflorescence structure and flower quality.

1. Introduction

Irradiance plays a key role in perennial fruit crop flower formation via directly or indirectly impacting photosynthesis, carbohydrate availability and resource partitioning, and also by modifying the internal chemical composition of the plant, particularly the balance of endogenous hormones, e.g. in apple (*Malus domestica* L.) (Jackson and Palmer, 1977; Lauri and Térouanne, 1999) and grape (*Vitis vinifera* L.) (Lebon et al., 2008; Petrie and Clingeleffer, 2005). Nevertheless, reported effects of irradiance on floral formation in the olive tree (*Olea europaea* L.) are scarce and seemingly inconsistent, with variable results in relation to the experimental method (artificial shadow and natural gradient) and the irradiance level used. Tombesi and Cartechini (1986), in adult trees, and Gregoriou et al. (2007), in young trees in containers, found that artificial shading up to 40% of daily incident photosynthetically active radiation (PAR) reduced inflorescence formation. Similarly, Acebedo et al. (2000), in a traditional olive orchard (density of 238 trees/ha), demonstrated that flower intensity and flower number

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per inflorescence increased in relation to more illuminated positions on the tree canopy. In contrast, Cherbiy-Hoffmann et al. (2015) observed that the intensity of artificial shadow (from 3 to 70% of daily incident PAR) and three shading periods during fruit set, pit hardening and oil synthesis did not affect the return flowering. Stutte and Martin (1986) did not find a relationship between various artificial irradiance levels and flowering in trees inside to growth chambers, mediated by inconsistent differences in carbohydrate levels between treatments.

Flower formation in the olive tree is complex, extending over two seasons. The interaction among the numerous micro- and macro-environmental conditions during this long period, previous fruiting behavior, genotype, and management practices give rise to considerable variation between seasons, trees in the same orchard and within the same tree (Acebedo et al., 2000; Cuevas et al., 1994). Flower development involves the formation of potential flowering buds in the leaf axils during spring-early autumn shoot growth, floral induction of those buds the following winter, and differentiation of the inflorescence and flower structures, which begins with bud break in late winter (i.e. 8–12 weeks before full bloom, WBFB) and finalizes by bloom (Cuevas et al., 1994; Reale et al., 2006). The period prior to bloom is critical for floral formation and thus fruit yield determination (Rapoport and Gómez-del-Campo, 2008). For example olive tree exposure to water stress from 10 weeks before full bloom to full bloom strongly affected different flowering parameters including inflorescence number, flower number, perfect flower number, and ovule development (Rapoport et al., 2012).

From the perspective of olive production, total flower number per olive tree represents an upper limit for olive orchard potential yield. However satisfactory production requires not only a sufficient number of inflorescences per tree and flowers per inflorescence, but these flowers must have good quality. Based on Williams (1965) characterization of floral structure and processes in apple, the term “floral quality” refers to any morphological or developmental characteristics of the flower that affect its ability to set and form a good quality fruit. Martins et al. (2006) extended this concept to olive and, in addition to the traditionally considered development of perfect (hermaphrodite) flowers in contrast to imperfect (staminate) flowers caused by varying degrees of pistil abortion (Cuevas and Polito, 2004; Reale et al., 2009), included additional parameters such as ovary size, which could influence fruit size, and ovule development, which affects potential fertilization and fruit set. Olive ovule development is often incomplete and an embryo sac is not formed, thus representing a possible limitation to fruit set. While usually only one ovule is fertilized for fruit set to occur, the lack of full differentiation of more than one of the four ovules present in the ovary is considered to reduce fruit-set capacity (Moreno-Alías et al., 2012). Consequently, potential fruit number in a particular canopy position can be represented by the product of the number of inflorescences x number of flowers per inflorescence x proportion of flowers of good quality (i.e. hermaphrodite with three or more developed ovules). Potential fruit size is related to floral attributes including ovary mesocarp and endocarp size, cell size and cell number (Rosati et al., 2012). Furthermore, developmental modifications in olive tree flowering due to total flower number (Cuevas et al., 1994) or water status (Rapoport et al., 2012) may lead to partial compensations among different flowering components, which could likely occur as well in relation to irradiance.

In recent decades, intensive hedgerow olive orchards have been established to facilitate mechanical harvesting which reduces the costs of manual labour and allows more rapid and timely management intervention (Connor et al., 2014). Hedgerow orchards planted at super-high density (more than 1000 trees/ha) are known to produce very high yield in early years after planting. This early yield advantage can, however, be lost with time if the growing canopy is not well illuminated (Trentacoste et al., 2015). Adequate hedgerow design and subsequent canopy management are required to avoid potential yield reduction in the mature olive hedgerows.

Irradiance effects on assimilate availability, oil synthesis and partitioning during fruit development, have recently been characterized in olive hedgerows (Castillo-Ruiz et al., 2015; Trentacoste et al., 2016). Knowledge of how inflorescence and flower development, the crucial first steps for fruit formation, respond to varied irradiance received at different positions on the hedgerow is also essential for improving hedgerow design, management and modeling. In this context the aims of this work were to (i) determine quantity and quality parameters of inflorescence, flower and ovary development at different canopy heights and orientations in olive hedgerow orchards and (ii) explore the relationships between the irradiance received in different periods and canopy positions, determined by simulation, and inflorescence and floral characteristics. We have also included closely related developmental events immediately prior and subsequent to flowering, in particular bud formation and fruit set, respectively.

2. Material and methods

2.1. Site and orchard

The study was carried out during 2013 in an olive hedgerow spacing experiment (Trentacoste et al., 2015) in an orchard (cv. Arbequina) planted in 2008 in La Puebla de Montalbán (39° N), Toledo, Spain. Two experimental plots, separated by approximately 100 m, were established, one with rows oriented N–S (North-South) and the other E–W (East-West). Each plot consisted of 3 rows of 48 trees spaced at 2.5 × 1.3 m, in which the central row was studied. Both hedgerow orientations had similar dimensions, i.e. canopy height 2.37 m and 2.27 m and canopy width 1.02 m and 1.10 m in N–S and E–W oriented hedgerows, respectively. Site, environment conditions and hedgerow management are fully described in Trentacoste et al. (2015).

2.2. Definition of canopy positions

Six individual olive trees were chosen randomly in each experimental plot (N–S and E–W hedgerows) among the 42 central trees in the central row (three for continuous evaluation of flower and fruit development and three for sampling of inflorescences and fruits). The canopy of each tree was divided into ten positions based on five vertical layers (heights) and two canopy sides or faces (Fig. 1 in Trentacoste et al., 2016). The designated layers for each side of the hedgerow were 0.0–0.4 m (layer 1), 0.4–0.8 m (layer 2), 0.8–1.2 m (layer 3), 1.2–1.6 m (layer 4) and 1.6–2.0 m (layer 5) above the soil surface.

2.3. Inflorescence and fruit formation

In winter, before budburst, three one-year-old shoots were selected at random for each position and tree, on which shoot length measured and bud number (node number x 2) counted. The number of inflorescences per shoot was recorded in the weeks prior to flowering (FB was June 4) and at 30 days after full bloom the number of fruits per shoot and inflorescences bearing at least one fruit (fruiting inflorescences). The measurements of the selected shoots were used to calculate the number of total buds which formed inflorescences on each one-year-old shoot, the percentage of buds forming inflorescences (floral buds), the percentage of inflorescence bearing at least one fruit (fruiting inflorescences), and the mean number of fruits per fruiting inflorescence. At harvest (29 October), total fruits of each layer and side were collected and counted. The shoot data were used along with the number of fruits per position in order to calculate the total inflorescence number per position as follows:

$$\textit{Total inflorescence per position} = \frac{\textit{fruit number per position}}{(\textit{fruit/fruiting inflorescence}) \times (\%\textit{fruiting inflorescence}/100)}$$

2.4. Inflorescence, flower and ovary characteristics

At flowering thirty inflorescences per layers 1, 3 and 5 were collected from the central zones of the flowering shoots, on both sides of the selected three trees for sampling in the two hedgerow plots. Sampling, conservation, and measurement of the inflorescences followed the procedures described by Moreno-Al & as et al. (2012). That is, inflorescences containing a mixture of open and closed flowers were immediately fixed and conserved until later measurement and subsampling of pistils (see Section 2.5). At that time inflorescence length and numbers of nodes, flowers and perfect flowers per inflorescence were counted and the percentage of perfect flowers was calculated.

Pistil histological preparation, observation, and measurement followed the procedures described by Moreno-Al & as et al. (2012) and Rosati et al. (2012). Ten pistils per layers 1 and 5 and both sides of each tree were selected from the perfect flowers of the sampled inflorescences, using a maximum of two pistils per inflorescence. As the timing of floral development within the olive inflorescence is not uniform (Rapoport and Gómez-del-Campo, 2008) and inflorescences containing both open and closed flowers had been collected, the pistils were taken from perfect flowers appearing to have opened most recently and thus best representing development at the time of anthesis. The pistils were processed according to standard paraffin procedure, transversely sectioned at 12 μm, and stained with toluidine blue O prior to paraffin removal (Sakai, 1973) for ovary evaluation and measurements.

The number of fully developed ovules of each ovary (10 per layer, side, and tree) was observed using an optical microscope (Leica DMRB-FHC, Leica Microsystems, Heerbrugg, Switzerland). The four ovules of each ovary were assessed and the ovaries were rated from 1/4 (one fully developed ovule of four) to 4/4 (four fully developed ovules of the four). No ovaries without any developed ovules were observed. Ovary, mesocarp and endocarp size were determined in 10 ovaries per position (layer and side) and tree, with a rating value of 3/4 or 4/4, that is ovaries considered to be of good quality (Martins et al., 2006); additional ovaries to the initially rated group were used if necessary to have ten which fulfilled this requirement. Images were captured with a digital camera (Leica DFC450C) and the ovary and its component tissues measured using an image analysis system (LAS v.4) connected to the optical microscope. Ovary and endocarp size were measured as the transverse area of the equatorial ovary section and mesocarp area was determined by subtracting endocarp area from total ovary area. For one repetition (tree), fifty mesocarp cells per ovary were counted and total area of the counted cells was measured to determine the average cell cross-sectional area. Total mesocarp cell number in the central transverse section was calculated from these data and total mesocarp area.

2.5. Irradiance values and evaluated periods

The model developed by Connor et al. (2016) was used to calculated mean daily horizontal irradiance (mol PAR/m^2) under clear-sky conditions for each layer and side. This model uses specific site and hedgerow parameters previously described in Trentacoste et al. (2015): latitude, date, hedge height, canopy width at base, row orientation, horizontal porosity, and row spacing. It operates daily at short intervals (10–15 min) to calculate solar position, irradiance beam, diffuse sky and reflected components, which it then uses to determine the irradiance value. The model performance was previously validated for the hedgerows studied in this experiment (Connor et al., 2016).

In order to explore the effect of irradiance during different floral developmental processes we selected specific short periods based on reproductive phenology (Rapoport et al., 2012). Period I (9 WBFB to 4 WBFB) represents the time of inflorescence formation and Period II (4 WBFB to FB) the final phase of floral development including pistil differentiation and gametogenesis. Period III (3 WBFB to 1 week after full bloom, WAFB) overlaps with Period II but extends one week after bloom in order to include pollination, fertilization and initial fruit set, the consequences of flower quality.

Daily incident irradiance on each canopy position (layer and side) was calculated using the model. Then mean annual daily irradiance values and mean daily irradiance for each of the chosen short periods of floral development and initial fruit set were obtained by averaging all daily values and the correlations between irradiance and the different flowering and fruiting parameters were tested for all periods.

2.6. Data analysis

ANOVA and the LSD mean separation test were used to test differences. Data of each hedgerow orientation were independently subjected to analysis of variance. The correlations between mean daily incident irradiance, and inflorescence and fruiting parameters were evaluated using Pearson's test, pooling together the values of the positions of both hedgerows. All statistical analyses were performed using the InfoStat 1.5 program.

3. Results

3.1. Irradiance profiles

In both hedgerow orientations the simulated mean daily irradiance increased progressively from period I to III at all positions and was higher than the annual average of daily irradiance (Table 1). Regardless of row orientation and period, the irradiance consistently followed a

Table 1
Simulated daily irradiance values (mol PAR/m^2) under clear-sky conditions for different periods at the studied hedgerow canopy heights (layers) and sides. Overall side and hedgerow daily irradiance are also shown.

Daily irradiance (mol PAR/m^2)								
	Period I (9 WBFB to 4 WBFB)		Period II (4 WBFB to FB)		Period III (3 WBFB to 1WAFB)		Annual (year 2013)	
N–S hedgerow layers	E	W	E	W	E	W	E	W
5	29.30	29.30	35.13	35.13	35.87	35.87	21.70	21.70
4	22.46	22.46	27.25	27.25	27.86	27.87	16.52	16.52
3	17.56	17.56	21.52	21.52	22.03	22.03	12.89	12.89
2	12.90	12.90	15.96	15.96	16.35	16.36	9.47	9.47
1	10.41	10.41	12.92	12.92	13.26	13.25	7.66	7.66
Side mean	18.53	18.53	22.56	22.56	23.08	23.08	13.65	13.65
Total mean	18.53		22.56		23.08		13.65	
E–W hedgerow layers	S	N	S	N	S	N	S	N
5	30.35	25.29	32.53	30.55	32.88	31.35	22.88	18.39
4	27.01	16.77	26.02	20.83	25.86	21.51	18.38	12.93
3	25.98	12.08	23.22	14.66	22.67	15.22	15.34	9.46
2	25.37	9.50	21.82	9.78	21.02	10.11	12.54	6.71
1	24.40	8.75	21.31	7.82	20.43	7.95	10.86	5.43
Side mean	26.62	14.48	24.98	16.73	24.57	17.23	16.00	10.58
Total mean	20.55		20.85		20.90		13.29	

Short periods I, II, and III were chosen in relation to floral development and initial fruit set.
Simulation based on model developed by Connor et al. (2016) as explained in the text.
Mean daily horizontal irradiance estimated values on bare field were 55.3, 64.5, 65.8, and 36.6 mol PAR/m^2 for Period I, II, III and annually, respectively.
Hedgerow layers 1, 2, 3, 4 and 5 correspond to hedgerow canopy heights 0.0–0.4 m, 0.4–0.8 m, 0.8–1.2 m, 1.2–1.6 m, and 1.6–2.0 m above soil, respectively.
WBFB: Weeks before full bloom. WAFB: Weeks after full bloom. FB: full bloom.

Table 2
Bud, flowering and fruiting parameters: number of axillary buds present on 1-year-old shoots in winter, percentage of those buds that developed an inflorescence (floral buds), percentage of inflorescences that set at least one fruit (fruiting inflorescence) and number of fruit per fruiting inflorescence at different heights (layers) and sides of N–S (E and W sides) and E–W (N and S sides) oriented olive hedgerows.

	Axillary buds/shoot (#)		Floral buds/shoot (%)		Fruiting inflorescence (%)		Fruit/fruiting inflorescence (#)	
N-S hedgerow layer	E	W	E	W	E	W	E	W
5	15.3 b	18.7 a	84.67 ab	69.25 c	58.33	39.58	1.4	1.6
4	15.3 b	13.8 bc	82.08 ab	76.92 bc	47.83	49.75	1.4	1.3
3	14.5 b	11.5 cd	85.33 ab	75.83 bc	54.67	58.50	1.3	1.5
2	13.2 bcd	11.5 cd	96.08 a	73.17 bc	46.50	55.42	1.3	1.4
1	12.7 bcd	10.7 d	79.17 bc	71.15 bc	44.58	45.77	1.4	1.4
E-W hedgerow layer	S	N	S	N	S	N	S	N
5	19.8a	19.8a	84.92	76.75	40.58	44.75	1.5	1.3
4	18.2abc	19.3ab	90.42	83.00	43.00	47.92	1.3	1.3
3	16.6bc	16.8bc	86.01	83.33	45.37	40.17	1.3	1.4
2	16.8bc	16.5c	88.42	86.75	46.50	45.50	1.3	1.3
1	17.0bc	12.7d	84.50	80.41	43.90	52.10	1.3	1.0

Hedgerow layers 1, 2, 3, 4 and 5 correspond to hedgerow canopy heights 0.0–0.4 m, 0.4–0.8 m, 0.8–1.2 m, 1.2–1.6 m, and 1.6–2.0 m above soil, respectively.
Values with the same letter are not significantly different among layers and sides (LSD test, $P \leq 0.05$). Letters only presented when ANOVA indicated significant effect.

general pattern of increasing from lowest to highest layer in the hedgerow. In the N–S oriented hedgerow both sides (E and W) received equal irradiance for each layer and irradiance in layer 5 was around 3 times greater than in layer 1 during all periods of simulation. In the E–W oriented hedgerow the S side had from 1.2 to 2.1 times greater irradiance in layer 5 than layer 1 during the different periods, the N side irradiance was around 3–4 times greater in layer 5 than layer 1. The S side received an average of 57% more total irradiance than the N side for all studied periods. For both hedgerow orientations the highest mean daily irradiance values were observed for Period III, which includes both final floral development before bloom and initial fruit set immediately after full bloom. During that period (III) the irradiance was highest on the S side (mean 24.6 mol PAR/m^2) of the E–W hedgerow, intermediate on the E and W sides (mean 23.1 mol PAR/m^2) of the N–S hedgerow, and lowest on the N side (mean 17.2 mol PAR/m^2) (Table 1).

3.2. Inflorescence formation and fruit set

Number of buds, flowering and fruiting parameters for the selected one-year-old shoots are shown in Table 2. Axillary bud number was highest for shoots in layer 5 and decreased with decreasing height for both sides of both hedgerow orientations. In the E–W hedgerow, the S side showed more axillary buds per shoot than N side, significantly for layer 1 by LSD ($P \leq 0.05$). No clear differences between sides were observed in the N–S hedgerow. With respect to the axillary buds that formed an inflorescence ("floral buds"), only minor differences were observed among layers within each of the four hedgerow sides and no tendency related to vertical height was observed (Table 2). Fruiting inflorescence percentage and fruit number per fruiting inflorescence did not vary significantly among positions nor show any tendency related to layer within the hedgerow sides of either hedgerow orientation (Table 2). In contrast, total inflorescence and fruit number of each layer and side varied with respect to canopy position, showing a vertical tendency of decreasing values toward lower layers (Table 3). In particular, inflorescence number and fruit number per layer were notably higher in layer 5 and sometimes layer 4 than in the three lower layers for both sides of the N–S hedgerow and the S side of the E–W hedgerow. With respect to the opposite sides of each hedgerow, in the N–S hedgerow no significant differences in inflorescence and fruit number were observed between opposing sides at each height. In contrast the E–W hedgerow S side bore significantly more inflorescences (1.6-fold) and fruit number (1.5-fold) than the N side for the two upper layers (5 and 4) (Table 3).

3.3. Inflorescence, flower and ovary characteristics

In both N–S and E–W hedgerows, inflorescence structure and flower quality were significantly affected by height in the canopy, generally showing higher values of length, number of nodes, flowers and perfect flowers from upper to lower layers of each hedgerow side (Table 4). With respect to hedgerow side, in the N–S hedgerow values were higher for the E side than W side, particularly in the uppermost layer (5), and in the E–W hedgerow the highest values were observed in the N side, with the exception of the percentage of perfect flowers, which was

Table 3
Total inflorescence and fruit number at different heights (layers) and sides of olive hedgerows oriented N–S (E and W sides) and E–W (S and N sides).

Hedgerow layers	N–S hedgerow				E–W hedgerow			
	Inflorescences per position (#)		Fruits per position (#)		Inflorescences per position (#)		Fruits per position (#)	
	E	W	E	W	S	N	S	N
5	1373ab	1637a	1121a	1037ab	1510a	949bc	922a	554c
4	1033bc	1244ab	693abc	804abc	1542a	1005bc	862ab	626bc
3	550de	639cd	391cd	562bcd	1097bc	1233ab	593bc	694abc
2	597cd	408de	361cd	317cd	789c	833c	478c	493c
1	112e	111e	70d	66d	361d	388d	168d	164d

Hedgerow layers 1, 2, 3, 4 and 5 correspond to hedgerow canopy heights 0.0–0.4 m, 0.4–0.8 m, 0.8–1.2 m, 1.2–1.6 m, and 1.6–2.0 m above soil, respectively.
Values are average of 3 trees. Values with the same letter are not significantly different among layers and sides of each hedgerow by LSD test, $P \leq 0.05$.

Table 4
Inflorescence parameters for different heights (layers) and sides olive hedgerows oriented N–S (E and W sides) and E–W (S and N sides).

Hedgerow layers	Length (mm)		Node number		Total flower number		Perfect flower number		Perfect flowers (%)	
	N–S hedgerow (E & W sides)									
	E	W	E	W	E	W	E	W	E	W
5	28.42a	25.30b	5.42a	5.21b	20.74a	19.06b	10.57a	8.49b	54.5a	46.0b
3	24.30b	21.73c	5.17b	4.76c	18.16bc	15.98d	6.65 cd	5.50d	37.3 cd	34.9d
1	23.68b	20.92c	4.91c	4.84c	17.00 cd	16.40d	6.19 cd	6.97c	35.2d	43.4bc
	E–W hedgerow (S & N sides)									
	S	N	S	N	S	N	S	N	S	N
5	25.66c	29.48a	5.23b	5.51a	17.97b	20.58a	12.94ab	13.31a	74.3a	64.7bc
3	26.28bc	26.22bc	5.10b	5.17b	17.14b	17.58b	11.21c	9.59d	65.0c	56.1d
1	27.50b	26.35bc	5.16b	5.12b	17.63b	16.72b	12.00bc	9.20d	70.8ab	54.3d

Hedgerow layers 1, 3 and 5 correspond to hedgerow canopy heights 0.0–0.4 m, 0.8–1.2 m, and 1.6–2.0 m above soil, respectively. Values with the same letter are not significantly different among layers and sides of each hedgerow by LSD test, $P \leq 0.05$.

higher in the S side. Additionally, relationships were found between inflorescence quality parameters which might increase potential fruit set, and the number of fruiting inflorescences per shoot, used as an index of fruit set (Fig. 1). That is, fruiting inflorescence number per shoot was closely associated with increased inflorescence length ($R^2 = 0.60$, $P < 0.01$, Fig. 1A), node number per inflorescence ($R^2 = 0.70$, $P < 0.001$, Fig. 1B), and flower number per inflorescence ($R^2 = 0.60$, $P < 0.01$, Fig. 1C), and more weakly with perfect flower number per inflorescence ($R^2 = 0.35$, $P < 0.05$, Fig. 1D).

Ovule development in ovaries located at different hedgerow positions showed minor differences in both the proportion of developed ovules and ovaries with at least three fully developed ovules (Table 5). Furthermore, a very high proportion (between 93 and 100%) of ovaries was well-developed in all studied hedgerow positions.

Ovary, mesocarp and endocarp sizes in layers 5 (top) and 1 (bottom), measured as equatorial transverse area presented in Table 6. Ovary size in the N–S hedgerow was greatest in E-side layer 5 and smaller but similar among E-side layer 1 and both W-side layers. In the E–W hedgerow ovaries were largest in both S-side layers, intermediate in N-side layer 5, and smallest in N-side layer 1. Differences among positions in each hedgerow were more pronounced for the endocarp than the mesocarp, and endocarp differences were analogous to those of the complete ovary. Within the ovary mesocarp, no cell number differences were observed among positions in either hedgerow while cell size was significantly lower for the bottom layer (1) in the E–W hedgerow (Table 7).

3.4. Relationships of flowering and fruiting parameters with irradiance

Among flowering parameters the correlations for node number, flower number, and total number of inflorescences with irradiance were significant for the three selected periods of simulated irradiance and annually (Table 8). In contrast the percentage of floral buds/shoot, percent perfect flowers/inflorescence, and inflorescence length did not significantly correlate with the simulated irradiance received in any period. Regarding fruiting parameters, the number of fruits per fruiting

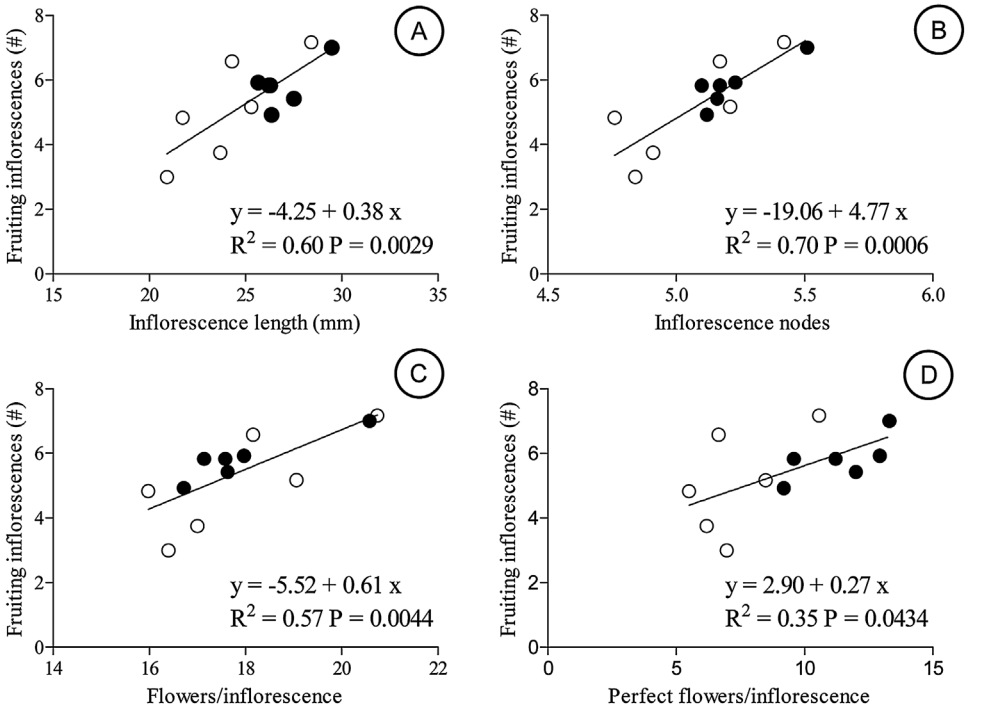

Fig. 1. Relationships between number of fruiting inflorescences i.e., inflorescences bearing at least one fruit and different inflorescence parameters: length (A), node number per inflorescence (B), total flower number per inflorescence (C) and perfect flower number per inflorescence (D), for selected shoots in layers 1 (0–0.4 m canopy height), 3 (0.8–1.2 m canopy height) and 5 (1.6–2.0 m canopy height) of both sides of N–S (empty circles) and E–W (solid circles) hedgerows.

Table 5
Proportions of developed ovules for ovaries of olive flowers at different heights (layers) and sides olive hedgerows oriented N–S (E and W sides) and E–W (S and N sides).

	Ovaries with different proportions of developed ovules (%)								Ovaries with ≥3 developed ovules (%)	
	4/4		3/4		2/4		1/4			
Hedgerow layer	N–S hedgerow									
	E	W	E	W	E	W	E	W	E	W
5	90.0	80.0	6.7	16.7	3.3	3.3	0	0	96.7	96.7
1	85.7	80.0	14.3	16.7	0	0	0	3.3	100	96.7
Hedgerow layer	E–W hedgerow									
	S	N	S	N	S	N	S	N	S	N
5	70.0	73.3	23.3	26.7	6.7a	0 b	0	0	93.3b	100a
1	83.3	74.8	16.7	25.2	0 b	0 b	0	0	100a	100a

Hedgerow layers 1 and 5 correspond to hedgerow canopy heights 0.0–0.4 m, and 1.6–2.0 m above soil, respectively.
The number of developed ovules (X) of the four total ovules per ovary is presented as a fraction (X/4). Ovaries with 3 or 4 ovules (final column) are considered to have good potential for fertilization and fruit set. Mean values of 30 ovaries (10 ovaries/layer/side/tree, in 3 trees).
Values with the same letter are not significantly different among layers and sides of each hedgerow orientation by LSD test, $P \leq 0.05$. Letters only presented when ANOVA indicated significant effect.

inflorescence correlated with irradiance for periods II, III, and annually, and total fruit number per position was highly correlated with simulated daily irradiance for all periods. No correlation was observed in any period for the percentage of fruiting inflorescences per shoot and the irradiance (Table 8).

4. Discussion

4.1. Bud formation, flowering and inflorescence number

The number of axillary buds per shoot, the first step in inflorescence formation, showed a clear pattern of descending values from upper to lower layers (Table 2), parallel to the pattern of descending irradiance received (Table 1). This finding is consistent with results by Acebedo et al. (2000) and Pastor et al. (2007) where more illuminated canopy positions showed higher shoot growth in both length and node number. Similarly, Cherbiy-Hoffmann et al. (2015) observed a close relationship between elongation of non-fruiting shoots and irradiance under artificially produced shadow levels (3, 20, 40 and 70% of transmittance), noting that shoot elongation increased linearly with irradiance up to a threshold of 40% of incident PAR, above which no further increase occurred.

The percentage of floral buds ranged 70–96% of the axillary buds

Table 6
Transverse area (mm^2) of ovary, mesocarp and endocarp for olive flowers at different heights (layers) and sides olive hedgerows (E and W sides of N–S hedgerow, N and S sides of E–W hedgerow).

Hedgerow layers	N–S hedgerow						E–W hedgerow					
	Ovary (mm^2)		Mesocarp (mm^2)		Endocarp (mm^2)		Ovary (mm^2)		Mesocarp (mm^2)		Endocarp (mm^2)	
	E	W	E	W	E	W	S	N	S	N	S	N
5	0.70a	0.65b	0.27	0.25	0.44a	0.40b	0.81a	0.76b	0.33a	0.32a	0.48a	0.44b
1	0.65b	0.66b	0.25	0.26	0.40b	0.40b	0.82a	0.69c	0.33a	0.28b	0.49a	0.40c

Hedgerow layers 1 and 5 correspond to hedgerow canopy heights 0.0–0.4 m, and 1.6–2.0 m above soil, respectively.
Mean values of 30 ovaries (10 ovaries/layer/side/tree, in 3 trees).
Values with the same letter are not significantly different among layers and sides of each hedgerow orientation by LSD test, $P \leq 0.05$. Letters only presented when ANOVA indicated significant effect.

Table 7
Cell size and total cell number for the mesocarp of ovaries from flowers at different heights and sides olive hedgerows (E and W sides of N–S hedgerow, N and S sides of E–W hedgerow).

Hedgerow layers	N–S hedgerow				E–W hedgerow			
	Mesocarp				Mesocarp			
	Total cell number		1 cell area (μm^2)		Total cell number		1 cell area (μm^2)	
	E	W	E	W	S	N	S	N
5	2210	2140	123	120	2972	2994	113a	110a
1	2404	2380	112	113	2826	2638	113a	96b

Hedgerow layers 1 and 5 correspond to hedgerow heights 0.0–0.4 m, and 1.6–2.0 m above soil, respectively.
Mean values of 10 ovaries/layer and 50 cells/ovary.
Values with the same letter are not significantly different among layers and sides of each hedgerow orientation by LSD test, $P \leq 0.05$. Letters only presented when ANOVA indicated significant effect.

Table 8
Correlation of flowering and fruiting parameters with mean daily irradiance (mol PAR/m^2) per position during different short periods related to flowering phenology and for the full annual period among all studied N–S and E–W olive hedgerow positions (layers, sides and orientations).

		9 WBFB to 4 WBFB (Period I)	4 WBFB to FB (Period II)	3 WBFB to 1WAFB* (Period III)	Annual
		r	*r*	*r*	*r*
Flowering parameters	Floral buds/shoot (%)	ns	ns	ns	ns
	Inflorescence length (mm)	ns	ns	ns	ns
	Nodes/inflorescence (#)	0.61*	0.62*	0.64*	0.61*
	Flowers/inflorescence (#)	0.65*	0.75**	0.76**	0.70*
	Perfect flowers/inflorescence (%)	ns	ns	ns	ns
	Inflorescences/position (#)	0.71***	0.74***	0.75***	0.82***
Fruiting parameters	Fruiting inflorescences/shoot (%)	ns	ns	ns	ns
	Fruits/fruiting inflorescence	ns	0.50*	0.51*	0.49*
	Fruits/position (#)	0.72***	0.80***	0.81***	0.86***

WBFB: Week before full bloom. WAFB: Week after full bloom. FB: Full bloom.
ns: correlation not significant $P > 0.05$ (Pearson).*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

per shoot and was affected only slightly by shoot position within hedgerows (Table 2). Nor was there a correlation of floral bud percentage per shoot with daily irradiance received in any of the short periods or annually (Table 8). Consequently the number of inflorescences per shoot followed trends similar to those of number of axillary buds per shoot, decreasing toward the less illuminated lower canopy layers (Table 2). In shading and defoliation experiments Proietti and Tombesi (1996) concluded that although olive buds require assimilates for differentiation they are a relatively weak sink. Mert et al. (2013) found that the onset date of olive flower bud differentiation was not influenced by “on” or “off” bearing year, indicating that assimilate was not limiting for floral differentiation under their conditions. In our study the quantity of irradiance received in the bottom canopy layer 1, representing from 15 to 30% of horizontally incident PAR in the annual period (Table 1), also appeared to be sufficient to allow adequate floral induction, so a negative effect of reduced irradiance did not occur. Another reason for no observed irradiance effect on floral induction could be that the flowering stimulus could be translocated from more illuminated to poorly illuminated canopy positions, as suggested in olive by Proietti and Tombesi (1996) and Fabbri and Benelli (2000). Nonetheless the uncertain role of irradiance as a flowering stimulus (Thomas, 2006) remains unresolved and the bud-induction response to irradiance requires more studies.

Clear differences among layers were seen in total inflorescence number per position, which decreased dramatically from upper to lower layers in each hedgerow (Table 3) and correlated strongly with irradiance received (Table 8). Dag et al. (2010) demonstrated a strong competitive effect of developing fruits with new shoot growth necessary for forming potential flowering sites, similar to our observations for positions receiving reduced irradiance, and emphasized the critical nature of flowering site number in the olive tree bearing cycle. Since inflorescence number per shoot derives directly from bud number and percentage of floral buds, parameters which showed only minor differences (Table 2), the substantial differences in inflorescence number per layer were produced mainly by differences in total number of reproductive shoots per position, suggested previously by Trentacoste et al. (2015), with lesser shoot level contributions of bud number and percent of buds forming inflorescences.

4.2. Inflorescence growth and flower characteristics

Irradiance received in different hedgerow positions clearly affected flower number per inflorescence, with a significant tendency for increase in more illuminated positions, while inflorescence length and node number showed a lesser or null response (Table 4). Previous studies of olive tree inflorescence structure and flower number have shown limited and sometimes contradictory results. Seifi et al. (2011) found that although shoot orientation around the tree canopy did not affect either inflorescence length or flower number per inflorescence, positon along the shoot did, so that inflorescences at shoot tips and centers were longer and had more flowers than at the more shaded shoot bases. Nutritional studies by Erel et al. (2013) indicated an effect of nitrogen and phosphorous but not potassium on both inflorescence length flower number, but Fernández-Escobar et al. (2008) found no effect of nitrogen on these parameters.

Correlations of flowering parameters with simulated profiles of mean daily irradiance (Table 8) support the assumption that irradiance dependent assimilate synthesis by leaves influences the number of flowers on each inflorescence. Thus flower number per inflorescence was related to mean daily irradiance for the short simulated periods and annually, as was number of nodes per inflorescence, a structural trait closely associated with flower number. Inflorescence length, however, showed no relationship with simulated irradiance, perhaps because either assimilate wasn’t limiting for this parameter or reduced growth due to assimilate limitation in less illuminated positions was compensated by increased elongation due to mild etiolation.

Number and percentage of perfect (hermaphrodite) flowers increased in the higher and more illuminated positions, although those overall tendencies were sometimes broken for the lowest layer 1 (Table 4). In olive, pistil abortion producing imperfect flowers is determined by both genetic and physiological causes, with competition for assimilate being one of the critical physiological factors (Cuevas et al., 1994; Reale et al., 2009). Thus, in addition to the observed hedgerow-position related tendencies, one would also expect a correlation with irradiance, particularly in periods II and III, which coincide with the time of pistil differentiation (Cuevas et al., 1999), but that was not found (Table 8). On the other hand, our results showed similar changes in both total and perfect flowers associated with hedgerow position, in contrast to the usually observed lower or unchanged percentage of perfect flowers when flower number is higher (Baratta et al., 1986; Uriu, 1956) or an effect only on perfect but not total flowers (Levin and Lavee, 2005), but also found by Erel et al. (2013) in response to nutritional status. The dual response of both total and perfect flowers to position in the hedgerow indicates little competition for substrates between these parameters, consistent with sustained differences in photosynthesis among positions receiving different irradiance.

With respect to ovule development in the ovaries of the perfect flowers, an absolute requirement for fertilization and fruit set, all studied positions presented flowers with high quality ovaries, with 93% or more presenting 3 or 4 fully developed ovules (Table 5). Cuevas et al. (1994) found no fruit load effect on ovule development, but increased ovule longevity with lower crop load and less competition for assimilates. Good ovule development appears to be a cultivar characteristic of ‘Arbequina’ (Moreno-Alías et al., 2013) which positional irradiance differences did not affect, suggesting this cultivar characteristic is a useful choice for hedgerows.

4.3. Flowering, fruit number and position irradiance

Inflorescence and flower development are comprised by multiple sequential processes which irradiance likely affects to different degrees. Then, as flowering progresses to fruit set, any hedgerow-position irradiance effects on the capacity to form a fruit may contribute to final fruit number. These responses are expressed at the branch level, that is physiologically within the fruiting branch, and at the canopy position (layer and orientation) or global level.

At the branch level the number of fruiting inflorescences related positively to the different flowering parameters but to varying degrees of significance (Fig. 1). Very likely there was some compensation among components, such has been observed due to water status (Rapoport et al., 2012). Other processes involved in determining fruit set, such as pollination and fertilization, and other environmental factors, such as temperature and the timing of irradiance could be involved. Also the high sink priority for flower development may buffer irradiance differences. Still, there was an overall higher fruit set toward more illuminated canopy positions which confirms the importance of hedgerow orchard design and management to maximize irradiance received (Connor et al., 2014). Additionally, higher fruiting inflorescence number toward more illuminated canopy positions with a higher inflorescence number (Table 3), suggests that assimilates production was not limiting for fruit set.

Considering the fruiting shoots individually, flower quality as affected by irradiance was related to fruit number. However when the total population of fruiting shoots in each position is taken into account, the irradiance-based differences in overall fruit number are considerably greater and indicate a very strong dependence on the number of potential flower sites produced, i.e. the buds formed in the leaf nodes of the shoots. While there was a noticeable relation of buds produced per shoot (Table 2), indicating positional differences in individual shoot growth, there was also a substantial position effect on shoot number (Trentacoste el al., 2015), which together produced very pronounced differences in inflorescence and fruit numbers per position (Table 3). When correlated with calculated mean daily irradiance, fruit number showed a strongly positive relationship for all studied periods, with the best correlation for mean annual values (Table 8). Fruit removal experiments by Dag et al. (2010) concur with the importance of assimilate-influenced vegetative growth of flowering sites in determining final fruit production.

4.4. Ovary growth and possible consequences for fruit size

Ovary size, indicated by transverse area, was either similar or greater in top than bottom layers of all hedgerow sides and differed between E and W sides in the N–S oriented hedgerow (Table 6). The ovary differences among positions were due to those shown by the endocarp, which occupies approximately 60% of the olive ovary at bloom. The endocarp is considered to be a relatively strong sink within the fruit due to its evolutionary role in protecting the seed (Hammami et al., 2013), sink capacity which was reflected in the relatively uniform endocarp size of mature fruits growing at same layers and hedgerows used in this study, even though overall fruit size was clearly affected (Trentacoste et al., 2016). It appears that the ovary endocarp at bloom was limited by low assimilate level in the less illuminated positions, but full endocarp growth and sclerification still took place during fruit growth, although more slowly than in more illuminated positions due to strong sink capacity, as has been observed when water is limiting (Hammami et al., 2013) and was hypothesized in our previous study (Trentacoste et al., 2016). The consequence of any delay in endocarp growth, either in the ovary or fruit, would be that later full endocarp development, including the metabolically expensive sclerification process, would be a competing sink to fruit mesocarp (pulp) growth and oil production.

The ovary mesocarp showed almost no significant difference among positions, either in size (Table 6), or cell size and number (Table 7). This contrasts with the close and positive relationship between irradiance and fruit size at harvest observed in previous studies conducted in the same olive hedgerows (Connor et al., 2016; Trentacoste et al., 2016), and by observations of Rosati et al. (2012) that among cultivars of different fruit size pre-anthesis cell division exerted an important influence on potential fruit size. However it appears that ovaries in all positions had similar potential for growth and that fruit size differences among positions were mainly determined by irradiance differences during fruit growth.

5. Conclusions

We found that ovaries of the perfect flowers had a high number of developed ovules (3 or 4), regardless of inflorescence position within canopy, while the number of perfect flowers per inflorescence increased toward more illuminated canopy positions. Thus at the inflorescence level, assimilates required for the formation of perfect flowers were also sufficient to produce good ovule development. In addition, ovaries of inflorescences in more illuminated positions showed greater size than those of less illuminated positions, largely due to a relative higher sink priority of the endocarp than the mesocarp during ovary development.

At the branch level, fruiting shoot position within canopy significantly influenced inflorescence characteristics: inflorescences on more illuminated shoots had more total flowers and perfect flowers than on less illuminated shoots, and thus greater potential for fruit formation. However fruit set, measured as fruiting inflorescence proportion per shoot and fruits per inflorescence, was little responsive to irradiance. This latter issue requires further study considering pollination, pistil receptivity and the ovule longevity response to both irradiance and temperature. It is also possible that in less favorable years, such as with environmental limitations, these parameters may be more critical.

At the canopy level, fruit number per layer increased linearly with irradiance, as did inflorescence number. Higher fruit number under greater irradiance was very strongly associated with increased number of potential flowering sites per position i.e. more branches and buds per shoot. This irradiance effect on vegetative growth causing more and longer fruiting shoots is previous to and much greater than the more direct influence of irradiance on flower development, which is present but secondary.

Our findings regarding flowering and its subsequent influence on fruiting at the inflorescence, branch and canopy levels reinforce the importance of irradiance level at different positions in olive hedgerows. Potential fruit number and thus production may be optimized by the adequate choice of row spacing and row orientation during orchard establishment and the management of canopy height and width by pruning as the plants grow and mature. The irradiance model (Connor et al., 2016) provides an efficient hedgerow design and management tool to determine irradiance parameters for different locations and environmental conditions.

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