Olive inflorescence and flower development as affected by irradiance received in different positions of an east-west hedgerow

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Abstract

Olive tree productivity is highly responsive to radiation conditions, so understanding the responses of the developmental processes that determine yield, including inflorescence and flower differentiation, is essential for orchard design and management. This is particularly true in the new intensive hedgerow orchards, where radiation reception highly depends on canopy height and row orientation and spacing. In an east-west-oriented olive 'Arbequina' hedgerow located in Toledo (Spain), inflorescences were sampled from both sides (south and north) of the hedgerow canopy, at heights of 0-0.4, 0.8-1.2 and 1.6-2.0 m above the soil. Floral quality was determined at different levels of morphogenetic organization: inflorescence, flower, and ovary. Daily irradiance intercepted during 1 month before flowering by each canopy position was estimated using a model. The more highly illuminated south side received 31% more irradiance overall than the north side. Upper-layer irradiance was greater than the bottom layer, 4.0 and 1.5 times for the north and south sides, respectively. Inflorescence flower number and perfect flower proportion were similar at different heights on the south side. In contrast, north-side upper-layer inflorescences had more total flowers and perfect flowers than at lower hedgerow heights. At each height, perfect flower proportion was higher on the south than north side, while the remaining traits were similar between sides. Ovary tissue size, observed in histological preparations, did not vary among heights on each side, but was higher on the south than north side due to endocarp size. Simulated irradiance at flowering explained 90% of the observed variation of perfect flower number per inflorescence. Overall, the results emphasize the importance of irradiance at different hedgerow sides and heights on olive floral structures.

Keywords: *Olea europaea*, floral quality, flower number, ovary size, irradiance model

INTRODUCTION

In the olive tree, reported effects of irradiance on floral formation are scarce and furthermore seem to vary with respect to the irradiance levels used, and whether the irradiance levels were produced experimentally by artificial shading or by natural gradients in the canopy. Tombesi and Cartechini (1986), in adult trees, and Gregoriou et al. (2007), in young trees in containers, reported that artificially shading up to 40% of daily incident photosynthetically active radiation (PAR) reduced inflorescence formation. Similarly, Acebedo et al. (2000), in a traditional olive orchard, demonstrated that flower intensity increased in the more illuminated positions of the tree canopy. In contrast, Cherbiy-Hoffmann et al. (2015) observed that the intensity of artificial shading during fruit set, endocarp sclerification and oil synthesis did not affect return flowering, and Stutte and

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Martin (1986) found no relation among various artificial irradiance levels and flowering in trees in growth chambers.

Total flower number per olive tree represents an important limitation for potential yield from an olive orchard. Adequate fruit production, however, requires not only a large number of inflorescences per tree and flowers per inflorescence, but also that these flowers must have good quality, that is, characteristics that affect their ability to set fruit. Important flower quality parameters in the olive tree include aspects of ovary and ovule differentiation, as well as the traditionally evaluated development of perfect (hermaphrodite) flowers in contrast to imperfect (staminate) flower formation, resulting from varying degrees of pistil abortion (Martins et al., 2006). Furthermore, modifications early in flower development may lead to compensatory changes later, for example canopy positions with fewer inflorescences may show larger numbers of flowers per inflorescence and/or better floral quality (Lavee et al., 1996).

In recent decades, intensive hedgerow olive orchards have been established to facilitate mechanical harvesting, reducing the costs of manual labour and allowing more rapid and timely management interventions (Connor et al., 2014). These intensive hedgerow orchards are known for their high yields in the early years after planting (León et al., 2007). This early yield advantage can, however, be lost with time, as trees fill their allotted space and light limitation intensifies (Trentacoste et al., 2015). To avoid potential yield reduction in the mature olive hedgerows, adequate hedgerow design and subsequent canopy management are needed.

Irradiance effects linked to patterns of assimilate availability and partitioning on fruit formation have recently been studied in olive, including the key steps of oil synthesis (Trentacoste et al., 2016). Little is known about the influence of irradiance on inflorescence and flower formation, however, critical early steps for determining fruit number and consequently hedgerow productivity. This knowledge could be highly useful to improve hedgerow design, management and modelling. In this context, the aims of this work were (i) to determine quantity and quality parameters of olive inflorescence, flower and ovary development at different canopy positions in an east-west-oriented olive hedgerow, and (ii) to explore the relationship of simulated irradiance received during floral development in each canopy position with those parameters.

MATERIAL AND METHODS

Site and orchard

The study was carried out during 2013 in an olive hedgerow ('Arbequina') oriented east-west (E-W) and planted in 2008 near La Puebla de Montalbán (39°N), Spain. The plot consisted of three rows of 48 trees spaced at 2.5×1.3 m, in which three individual olive trees were chosen randomly from the central row. For sampling and measurements, the canopy of each tree was divided into three heights (0.0-0.4, 0.8-1.2 and 1.6-2.0 m above ground) on both the south (S) and north (N) sides of the hedgerow.

Inflorescence, flower and ovary characteristics

Inflorescence sampling and processing, and ovary preparation, observation and measurement, followed the procedures used by Martins et al. (2006) and Moreno-Alías et al. (2013). At bloom (4 June 2013), 30 inflorescences per tree containing a mixture of open and closed flowers were sampled and preserved until processing. Number of flowers per inflorescence and number and percentage of perfect flowers were determined for all three heights of each canopy side. For the top and bottom positions, 30 pistils per tree were obtained from the inflorescence samples, utilizing a maximum of two pistils per inflorescence. The pistils were processed according to a standard paraffin procedure (Ruzin, 1999), sectioned transversely at 12 µm, and stained with toluidine blue O prior to paraffin removal (Sakai, 1973) for ovary tissue measurements and ovule evaluation.

The ovules of ten ovaries per position were observed and characterized as having normal or anomalous development. Based on the four ovules present in the olive ovary, the ovaries were scored according to the proportion of normal, fully developed ovules of the four, that is $x/4$, with x presenting values from 1 (one fully developed ovule) to 4 (four fully developed ovules). Subsequently, ovary and ovary tissues were measured for ten ovaries that had either three (3/4) or four (4/4) fully developed ovules, that is ovaries considered to have sufficiently good development for fertilization and fruit set (Martins et al., 2006; Rapoport et al., 2012). Transverse areas of ovary tissues were measured at the point of widest ovary diameter using image analysis (LAS version 1.4) connected to an optical microscope (Leica DMRB-FHC; Leica Microsystems, Heerbrugg, Switzerland) and digital camera (Leica DFC450C). Total ovary area and endocarp areas were measured, and mesocarp area was determined by subtracting endocarp area from ovary area (Martins et al., 2006; Moreno-Alías et al., 2013; Rapoport et al., 2012).

Irradiance values

A 30-day pre-flowering period (5 May to 4 June) was considered to represent flower development and flowering for irradiance value calculations. The model developed by Connor et al. (2016) was used to calculate daily incident irradiance (mol PAR m^{-2}) on each canopy position over the indicated 30-day period by averaging all daily values for that period. This model uses specific site and hedgerow parameters: latitude, day of year, hedge height, canopy width at base, row orientation, horizontal porosity and row spacing, previously described by Trentacoste et al. (2015). It operates daily at short (10-15 min) intervals to calculate solar position, beam irradiance, diffuse sky and reflected components, which it then uses to determine the irradiance. Model performance was previously validated in the hedgerows studied in this work (Connor et al., 2016).

Data analysis

Data were subjected to analysis of variance, and the least significant difference (LSD) test ($P<0.05$) was used to separate means of evaluated traits. Relationships between mean daily incident irradiance and inflorescence parameters were explored by linear regression analysis. All statistical analyses were performed using the InfoStat 1.5 program.

RESULTS

Irradiance profiles

The mean daily irradiance in the studied positions (two sides and three heights) of the E-W hedgerow is presented in Table 1. The irradiance followed a general pattern, where irradiance was greatest at the upper positions and decreased in successively lower canopy positions. The S side had 1.5 times more irradiance in the upper than the bottom layer around full bloom. On the N side, the irradiance on the upper layers was 4.0 times greater than at the bottom. Additionally, the S side received 31% more irradiance than the N side during the flowering period.

Table 1. Average daily irradiance (mol PAR m^{-2}) during 1 month before full bloom (5 May to 4 June) and inflorescence parameters at different heights and sides (S and N) of an E-W-oriented olive hedgerow.

Height in hedgerow (m)	Daily irradiance (mol PAR m^{-2})		Flowers per inflorescence		Perfect flowers per inflorescence		Perfect flowers (%)	
	S	N	S	N	S	N	S	N
1.6-2.0	32.53	30.55	17.97b	20.58a	12.94ab	13.31a	74.3a	64.7bc
0.8-1.2	23.22	14.66	17.14b	17.58b	11.21c	9.59d	65.0c	56.1d
0.0-0.4	21.31	7.82	17.63b	16.72b	12.00bc	9.20d	70.8ab	54.3d

Values with the same letter are not significantly different among positions (heights and faces) of the hedgerow by LSD test at P≤0.05.

Inflorescence, flower and ovary characteristics

Inflorescence flower quality was significantly affected by inflorescence position within the canopy. In general, values were greater in the higher positions and decreased toward the lower positions (Table 1). Inflorescence flower number and perfect flower proportion were similar at different heights on the S side. In contrast, N-side upper-layer inflorescences had more total flowers and perfect flowers than lower hedgerow heights. Between sides at each height, the perfect flower proportion was higher on the S than N, although inflorescence total flower number differed between sides only in the upper position and perfect flower number in the middle and bottom positions.

There were no differences among canopy positions, either for different sides or heights, in ovary quality. All positions had a very high proportion (consistently over 90%) of ovaries with at least three completely developed ovules, the threshold assumed to have a good chance for fertilization and fruit set (data not shown). The ovary equatorial transverse area did not vary between heights on each side, but was significantly higher on the S than N side. Those differences were due to the endocarp transverse area, but not that of the mesocarp.

Relationship of floral quality to irradiance

Relationships of flower quality parameters to mean daily irradiance simulated under clear-sky conditions over the 30-day period before flowering are presented in Figure 1. The number of perfect flowers per inflorescence was very strongly related to irradiance (R^2=0.91; P=0.005), and the relationship between flower number and irradiance, although not significant, also showed a positive tendency (R^2=0.43; P=0.12).

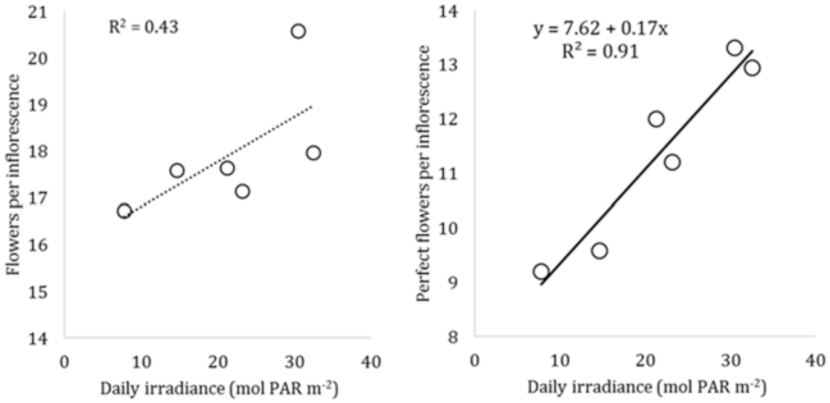

Figure 1. Relationships of inflorescence parameters and simulated daily average irradiance over the 30-day period before flowering. The solid line (right) shows regression fitted to the pooled data (P≤0.05); the dashed line (left) indicates the trend, although regression was not significant at P≤0.05.

DISCUSSION

The vertical distribution of fruit number along the different hedgerow sides was shown previously by Trentacoste et al. (2016). That study indicated that fruit number in an E-W-oriented hedgerow was highest in the uppermost canopy positions, and decreased toward the base, consistent with mean annual daily irradiance distribution. Here, the differences observed for the various floral parameters demonstrate that irradiance differentially affected the multiple sequential processes that determine fruit number distribution.

Inflorescence, flower and ovary characteristics

It has been reported previously for the olive tree that the number of inflorescences that appear in spring depends mainly on shoot growth in the previous season, where fruit load is the main control (Lavee, 2006). The number of flowers per inflorescence, however, is highly affected by environmental conditions and available assimilates (Fernández-Escobar et al., 2008). In our study, the strong and positive relationships between measured flower numbers, in particular perfect flowers, per inflorescence and simulated profiles of mean daily irradiance the month preceding flowering support the assumption that assimilate synthesis by the nearby leaves plays an important role in controlling the number of flowers on each inflorescence (Table 1). Thus, from an olive management perspective, hedgerow design and also winter pruning tending to increase canopy illumination could mitigate year-to-year variations in inflorescence density widely observed in olive (Lavee, 2006).

In olive, a frequent cause of pistil abortion producing imperfect (staminate) flowers is competition among flowers for resources, which are insufficient when flowering is high (Perica et al., 2001; Levin and Lavee, 2005) or ovaries are larger (Rosati et al., 2011). Similarly, here we found that assimilate limitation by reduced irradiance affected the number of perfect flowers more than the flower number. Consequently, the proportion of perfect flowers was higher on the S than N side, and decreased downwards from upper to bottom layers on the N side, resembling the irradiance pattern (Table 1).

Ovaries in all positions presented good ovule development. Since only one ovule is required for fertilization and subsequent fruit set, three as well as four developed ovules is considered good quality for fruit formation (Martins et al., 2006). The lack of differences could be due to the generally high-quality ovule development in 'Arbequina' (Moreno-Alías et al., 2013) or a high sink priority within the ovary for ovule development (Rapoport et al., 2012).

Ovary tissue size was higher on the S than N side, apparently because of the larger endocarp. These results differed from previously published results indicating uniform fruit endocarp size at the same positions and on the same hedgerows (Trentacoste et al., 2016). We hypothesize that, in the less-illuminated (N side) positions, the ovary endocarp is initially limited by low assimilate levels, but full endocarp growth recovers and full size is reached later as photosynthesis is able to progress. The capacity to reach a full-size fruit endocarp in spite of a small ovary endocarp is probably related to the evolutionary importance of the endocarp for protecting the seed. Similar compensatory endocarp growth after previously reduced development has been observed during recovery from early water deficit (Gómez-del-Campo et al., 2014).

CONCLUSIONS

Using the modelled irradiance pattern during flower development, we observed potential links between irradiance in different positions in an E-W-oriented olive hedgerow and inflorescence, flower and ovary characteristics. Irradiance received on the canopy strongly affected the number of perfect flowers per inflorescence and ovary endocarp size, possibly because of the role of assimilate supply to reproductive organs. Ovule development and ovary mesocarp size were unrelated to irradiance. These results provide valuable information regarding how design (row orientation, spacing, hedgerow dimensions) and management practices that increase irradiance received by the canopy during flowering can influence olive hedgerow productivity.

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