Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology: Official Journal of the Societa Botanica Italiana

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tplb20

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Accepted author version posted online: 24 Oct 2014.Published online: 16 Dec 2014.

Plant Biosystems, 2014
Vol. 148, No. 6, 1221–1230, http://dx.doi.org/10.1080/11263504.2014.980360

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ORIGINAL ARTICLE

Influence of canopy fruit location on morphological, histochemical and biochemical changes in two oil olive cultivars

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Abstract
The influence of different irradiance conditions was evaluated under natural solar radiation by comparing well-exposed (in) and shaded fruit (out) in canopies of olive trees (*Olea europaea* L). Over a 2-year period, from 50 days after full bloom up to harvest time, "in" and "out" olive samples of two genotypes ("Frantoio Millennio" and "Coratina 5/19") were periodically collected. Morphological, histochemical, and biochemical analysis were performed to study the changes on fruit morphometric traits, oil body accumulation, and β-glucosidase enzyme activity. Some parameters were modified by shading inside the canopy in which the proportion of incident photosynthetically active radiation intercepted by the crop was 47%. Shaded fruits developed at slow rate and were characterized by late darkgoing time, reduced size, with a tendency toward oblong shape. The rapid histochemical procedure proposed to estimate the oil body accumulation during fruit ripening showed that a reduced irradiance caused a decrease in oil body density. The canopy position influenced, in a different way, the β-glucosidase activity in relation to the fruit-ripening stage in both genotypes. These findings indicate that providing an adequate and uniform lighting of the olive canopy by careful choices of orchard management practices can be a key factor for several yield components.

Keywords: *β-glucosidase, fruit growth, fruit location, oil body,* Olea europaea *L*

Introduction

In fruit trees, several agronomic parameters can modify the quality of fruits defined by pomological traits and concentration of certain metabolites. The most studied aspects include cultivar, fruit ripening, environmental conditions, and cultural practices (i.e. pruning and training systems). Published results on several fruit species, such as peach and apple, demonstrated that light environment close to the potential sites of fructification is the variable most strongly linked to spatial within-canopy variations in yield and fruit quality (Corelli-Grappadelli et al. 1994; Wünsche & Lakso 2000; Willaume et al. 2004). Similarly to these species, the yield of olive trees (*Olea europaea* L.) is influenced by the amount of photosynthetically active radiation (PAR) intercepted by the canopy (Villalobos et al. 2006). Experimental trials with natural shading showed a decrease in the number of inflorescences, fruit set, fruit size, and fruit oil content (Tombesi et al. 1999; Proietti et al. 2012a). Moreover, the seasonal development of the tree canopy influences radiation distribution in the tree and may affect leaf morphology and physiology (Gregoriou et al. 2007). These occurrences could be of interest considering the evolution of olive-cropping systems against new modern orchard managements with high planting density in which self-shading phenomena can easily occur determining the conditions of reduced irradiance (Proietti et al. 2012b).

The growth and olive fruit characteristics are significantly modified according to their position in vase-shaped olive canopies (Acebedo et al. 2000), and some differences are strongly related to the intercepted radiation (Connor et al. 2009). The effect of canopy position on fruit size, maturity, and oil content has been determined in "Arbequina" hedgerows, where greater values were recorded from the upper layers (Gòmez-del-Campo et al. 2009). Moreover, the fruit position modified some parameters used to evaluate the commercial and nutritional quality of the virgin oil, such as stability

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against oxidation, fatty acid composition, or phenol content (Gòmez-del-Campo et al. 2012).

Critical information is still lacking in order to predict how inadequate radiation affects the composition and biochemical status of olive fruits. The presence of hydrophilic phenols in virgin olive oil is directly related to the content of phenolic glycosides, initially present in fruit tissues, and to the activity of hydrolytic and oxidative enzymes (García-Rodríguez et al. 2011). Phenolic compounds have a direct influence as antioxidants providing important nutritional and health benefits, also impacting the sensorial and organoleptic properties of olive fruits and virgin olive oil (Perrin 1992; Baldioli et al. 1996; Romero-Segura et al. 2011). Phenolic composition, mainly determined by the content of phenolic glycosides (such as oleuropein and ligstroside) and aglycone derivatives, varies in qualitative and quantitative terms during the development and ripening process and may be affected by pre- and postharvest factors (Servili et al. 1999; Briante et al. 2002; Tovar et al. 2002). The hydrolysis of phenolic glycosides is promoted by endogenous glycosidases and, in particular, β-glucosidase exhibited maximum activity toward oleuropein (Romero-Segura et al. 2009). This compound is progressively degraded by the releasing of glucose and the aglycone molecules, with the consequent physiological debittering of fruit tissues (Ryan et al. 1999; Morello et al. 2004). Moreover, a positive correlation between β-glucosidase activity and high phenol concentrations in oil of cv. "Picual" cultivar was found, suggesting a critical role of β-glucosidase in shaping the phenolic profile of virgin oil (Romero-Segura et al. 2012).

The aim of this research was to investigate the effect of different light conditions under natural solar radiation by comparing well-exposed and shaded olive fruit at different stages of development. The changes on fruit morphometric traits, oil body accumulation, and β-glucosidase enzyme activity were studied.

Material and methods

Plant material and fruit sampling

Considering the alternate bearing habit of the olive (*Olea europaea* L.), the study was carried out over two "on" fruiting crop years (2010 and 2012) characterized by regular and high yields. Two Italian oil genotypes were considered: "Frantoio Millennio" (Frantoio M.), recently patented at Pisa University (NI724R/BI/rdv), and "Coratina 5/19", a "Coratina" cultivar's clone under evaluation (Table I). The experimental site was located at Scarlino (GR) in a hilly olive-growing area of central Italy (latitude 42°54′29″N, longitude 10°51′3″E, altitude 200 m above sea level). The orchard was managed under no irrigation and trees (10-year-old) were trained to vase system (6 m × 6 m) with a uniform vegetative growth. They have been pruned every year according to standard procedures and received routine conventional horticultural care. During the experimental trials, minimum and maximum daily temperatures and rainfall were acquired from ARSIA (agro meteorological service of Tuscany region).

Table I. Main traits of "Frantoio M." (Bartolini et al. 2013) and "Coratina 5/19" (Guerriero, pers. com.) Italian olive genotypes: geographical origin, darkgoing time, mean fruit weight, and diametric ratio (DR).

	Frantoio M.	Coratina 5/19
Geographical origin	Tuscany	Apulia
Tree vigor	Strong	Medium
Growth habit	Spreading	Upright
Canopy density	Medium	Medium
Leaf characteristics	Long, elongated	Long, very elongated
Flowering time	Medium	Medium
Darkgoing time	Medium	Extremely late
Fruit weight (g)	2.94 ± 0.19	1.57 ± 0.09
DR	1.34	1.38

In June, once fruit set started, four trees per cultivar were selected based on the similarity of tree size, number of branches, and crop load. To compare the fruit position in the canopy, the fruit-bearing branch and the fruit itself were chosen either at external side of the trees for the well-exposed fruit ("out"), or within the canopy for the shaded fruit ("in"). From July to September, the irradiance was estimated in the morning by measuring the PAR by a Ciras 1 Portable Photosynthesis System (HitChin, Hertfordshire, UK). At the internal side of the canopy, the incident radiation was 47%, with a reduction in maximum daily irradiance from about 1700 to 1500 $\mu E\,m^{-2}\,s^{-1}$ (full sunlight) to 750–650 $\mu E\,m^{-2}\,s^{-1}$ in July–August and September, respectively. The sunlight penetration (%) into the tree canopy was the average percentage of sunlight that reached the orchard floor and mid-canopy from the full sunlight.

"Out" and "in" fruits were periodically sampled and the following morphological, histochemical, and biochemical determinations performed.

Fruit growth and ripening. The morphometric and phenological changes of "out" and "in" fruits ($N = 50$ per canopy position) were assessed by fresh weight (g) of whole olive, pulp, and pit; flesh/pit ratio; calibration procedure dividing the olives into diametric classes; longitudinal and transverse diameters (mm) to determine the diameter ratio; and maturity index based on the degree of skin and pulp pigmentation according to Uceda and Frias (1975).

Histochemical localization of oil bodies. In order to assess the appearance and accumulation of oil bodies in the mesocarp cells, fruits were analyzed at (a) about 50 days after full bloom (DAFB, immature green fruit without woody endocarp); (b) about 120 DAFB, fully developed greenish-yellow fruits with woody endocarp; (c) about 160 DAFB, darkgoing fruits (green–brown spotted in "Frantoio M."); and (d) harvest time. In each year, full blooming time occurred during the third decade of May, without differences between the two genotypes.

From the point of widest diameter of fresh drupes ($N = 10$), sections of epi-mesocarp tissue at 30 μm of thickness were obtained by a vibratome (Vibratome 1000 PLUS Series 1000, Technical Products International, St. Louis, MO, USA). Sections were immediately floated on small drops of 70% ethanol solution of Sudan Black B on a clean glass slide. Sudan Black B staining was used for lipid identification following the procedure described by Bronner (1975) with modifications that consisted in a shortest staining time (5 min instead of 30 min), at room temperature instead of 60°C. Then, sections were washed twice with 70% ethyl alcohol and observed using a light microscope (Fluophot, Nikon Instrum. Inc., Melville, NY, USA). Representative selected sections were photographed with a digital camera (Photo-Bio, VWR International PBI Srl, Milan, Italy) equipped to the microscope. In a fixed area (50×10^{-3} mm^2), lipid bodies were counted and their diameter was evaluated as the average of the two orthogonal diameters measured using an image analysis software (Image tool).

β-Glucosidase activity. The β-glucosidase activity was performed on "out" and "in" fruits sampled at 50, 120, and 160 DAFB, according to Briante et al. (2002) and Mazzuca et al. (2006). Fresh pulp (1.0 g in three replicates) from fruits ($N = 10$ for each ripening stage) was extracted with 12.5 ml of borate buffer, 0.1 M pH 9.0 containing 5.0 mM EDTA (ethylendiaminetetraacetic acid), 1.0 mM PMSF (phenylmethylsulfonyl fluoride), and PVP (poly-vinilpirrolidone) 5% (w/v). The suspension was shaken gently for 1 h and centrifuged in a minifuge at 27,000*g* for 30 min. All operations were carried out at 4°C. The upper oil phase was removed and the aqueous phase, representing the active enzyme-enriched phase, was used for the enzyme assay. The amount of β-glucosidase activity was measured against *p*-D-nitrophenyl-β-D-glucopyranoside at 37°C by measuring the increase in absorbance at 405 nm of the reaction medium composed of 50 mM Na–phosphate buffer adjusted to pH 5.8. The linear coefficient used to calculate the concentration of the reaction product was measured using a calibration curve made with standard solutions of *p*-nitrophenol (Sigma-Aldrich Co., St. Louis, MO, USA) and corresponded to 14.0 M^{-1} cm^{-1}. The enzyme-specific activity was expressed as millimoles of *p*-nitrophenol produced per minute at 25°C per gram of fresh weight (mmol min^{-1} g^{-1}).

Statistical analysis

Data were processed for a completely randomized design, and the statistical analysis was performed using the Statgraphics Plus software ver. 5 (Manugistics, Inc., Rockville, MD, USA). The standard errors of the means (SEMs) were calculated for every parameter measured. Differences concerning data between the different locations of fruits in the canopy ("out" and "in") were tested by Student's *t*-test. A probability level of $p \leq 0.05$ was considered statistically significant. Analysis of variance (ANOVA) was performed and differences between means were considered statistically significant at $p \leq 0.05$ according to the Newman–Keuls test.

Results

Climatic conditions

The two crop seasons (2010 and 2012) were characterized by different summer conditions (Table II), mainly related to the distribution rather than the amount of rainfall that was comparable with the average of the previous 5-year period (250 ± 62 mm).

Table II. Monthly mean maximum and minimum temperatures (°C) and cumulative rainfall (mm) from June to October over two crop seasons (2010 and 2012).

	2010				2012			
	Temp. max	Temp. min	Temp. avg	Rainfall	Temp. max	Temp. min	Temp. avg	Rainfall
June	27.1	15.2	21.1	37.0	29.4	16.2	22.8	5.4
July	32.0	19.5	25.7	24.3	32.7	19.3	26.0	2.7
August	29.5	17.6	23.5	57.1	33.8	20.5	27.1	18.2
September	26.3	14.7	20.5	60.0	27.1	16.9	22.0	82.2
October	20.8	10.9	15.8	81.7	22.0	12.1	17.0	113.0
Total: June–August				118.4				26.3
Total amount				260.1				221.5

During the summer of 2012, mean temperatures were relatively warmer with very poor occasional rains from June to August. In this period, monthly rainfall was less than a quarter compared with the same time of year 2010, when rainfall events were 45% of the total (118.4 mm), similarly to the average of the previous 5-year period (80.2 ± 29.5). During the second crop season of 2012, about 88% of the total rainfall (195.2 mm) was concentrated between September and October.

Fruit growth and morphometric traits

The crop year and canopy position influenced the dynamic of fruit growth expressed as increase percentage on the basis of final fresh weight (Figure 1). While in the first year more than 50% of fruit weight was reached during July and August, in the same period of the second driest year a slower growth was recorded. Successively, the fruit growth rate was recovered but, at harvest time (November 8 and 15 in 2010 and 2012, respectively) a reduction in size was found in comparison with the first year. All the analyzed fruits had a mean equatorial diameter below 14 mm, independently to the canopy position (Table III).

Overall, the growth of "in" fruits was slowly and a mean fresh weight decrease of about 20% was recorded, with respect to "out" fruits (Figure 1). At harvest time (Table III), the "in" fruits showed low percentages of olives included in transverse diameter classes ≥ 14 mm, low values of maturity index, and a decrease in the flesh/pit ratio as a consequence of the reduction in pulp weight. Moreover, the "in" fruits were characterized by an increase in the diametric ratio, denoting a trend to a change in shape. Thus, the shape of these fruits was quite elongate in comparison with "out" fruits (Figure 2). Both genotypes differed for this trait namely in the second year when the shape of fruits appeared markedly elongated.

The ANOVA was carried out considering two main effects (canopy position and crop season) on the fresh weight and diametric ratio of fruits (Table IV). The ANOVA results indicated no significant interaction "canopy position × crop season" in both genotypes, although the fruit parameters analyzed

Table III. Transverse diameter (TD) class, maturity index (MI), flesh/pit ratio of fruits, and pit weight recorded at harvest time over two crop seasons (2010 and 2012) in samples from "out" and "in" canopy positions of "Frantoio M." and "Coratina 5/19" genotypes.

	Frantoio M.				Coratina 5/19			
	2010		2012		2010		2012	
	Out	In	Out	In	Out	In	Out	In
TD ≥ 14 mm (%)	63	40	0	0	17	7	0	0
MI	5.1	2.9[a]	0.7	0.5	2.8	1.7[a]	0.5	0.5
Flesh/pit	1.1	0.9[a]	1.6	1.5[a]	0.9	0.7[a]	2.3	1.8[a]
Pit weight (g)	1.1	1.2	0.6	0.6	0.8	0.9	0.4	0.4

[a] Significant differences between "out" and "in" olive fruits by Student's *t*-test ($p \leq 0.05$).

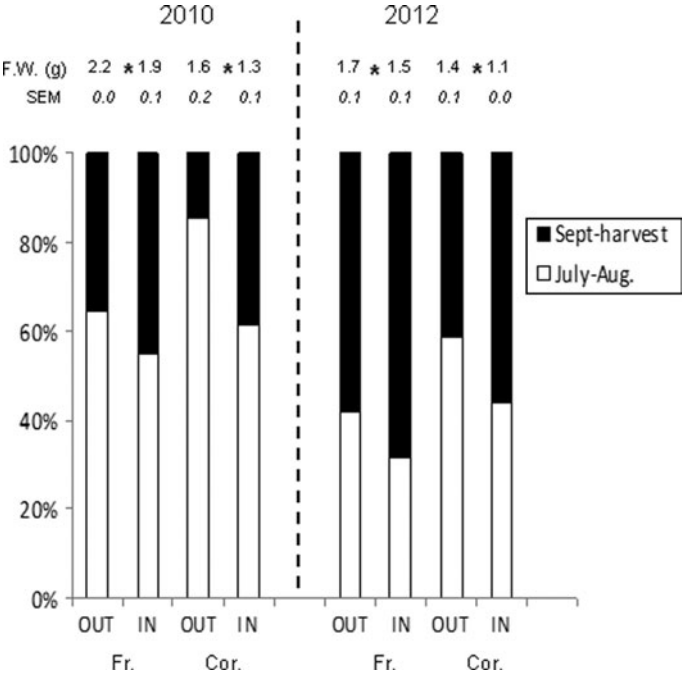

Figure 1. Percentage distribution of fruit weight detected over two crop seasons (2010 and 2012), from July to harvest in samples from external ("out") and internal ("in") canopy positions of "Frantoio M." (Fr.) and "Coratina 5/19" (Cor.) genotypes. At the top of bars, the final mean fresh weight of fruits is shown (±SEM). The asterisk (*) denotes significant differences between "out" and "in" olive fruits by Student's *t*-test ($p \leq 0.05$).

Table IV. *p*-Values resulting from two-way ANOVA.

	Frantoio M.	Coratina 5/19
Fruit weight		
Canopy position (CP)	0.0017	0.0157
Crop season (CS)	0.0010	0.1102 ns
CP × CS	0.9565 ns	0.2386 ns
Diametric ratio		
CP	0.0034	0.0183
CS	<0.0001	<0.0001
CP × CS	0.3120 ns	0.2878 ns

Notes: Main effect: canopy position and crop season. Interaction: canopy position × crop season. Variables: fresh weight and diametric ratio of fruits. ns, not significant.

were influenced by the shading and the crop season, in terms of summer environmental conditions.

Oil bodies

The histological analysis showed no significant differences in size and shape of mesocarp cells between cultivars. The simplified staining procedure for lipids allowed us to observe the presence and accumulation of oil bodies in mesocarp cells during different stages of fruit development.

In both genotypes, the presence of oil bodies becomes noticeable 50 DAFB (Figure 3(A)) when pit hardening took place in < 10% of examined fruits (data not shown). The oil body average diameter was < 15 μm, occupying 25–40% of the cell volume, considering that the cell mean diameter was 47.2 ± 6.0 μm (data not shown). At 120 DAFB (Figure 3(B)), when pit hardening was completed, the oil bodies had diameters of about 25 μm occupying more than 50% of the cell volume (cell mean diameter of 55.3 ± 5.0 μm, data not shown). From 160 DAFB to harvest time (Figure 3(C)), only one large oil body of 35–40 μm was usually detected, occupying 80% of the cell volume that was not different from those measured at the previous stage.

The dynamic of oil body accumulation, expressed as density in a fixed area (50×10^{-3} mm^2), and the diameter measurements were found to differ between genotypes and fruit canopy location, with a similar trend over the two considered crop seasons (Figure 4 (A)–(D)). "Coratina 5/19", from the early phenological stage of fruits (50 DAFB), showed the highest oil body density that decreased progressively, whereas oil body diameter increased regularly up to harvest time (Figure 4(A),(B)). An influence of fruit canopy location was observed: in mesocarp cells of "in" fruits the oil body number was always lesser than that of "out" fruits. This trend was also found analyzing the diametric measurements but only at the immature green fruit stage.

In "Frantoio M.", the oil body density increased from 50 to 120 DAFB and subsequently decreased up to harvest time, whereas the oil body diameter progressively increased from 50 DAFB to harvest time, similarly to "Coratina 5/19" (Figure 4(C),(D)). Generally, in "out" fruits, a higher density of oil body was observed, although in most cases there were no differences between "in" and "out" fruits.

β-Glucosidase activity

During olive fruit ripening, β-glucosidase activity of pulp extracts underwent significant changes with a

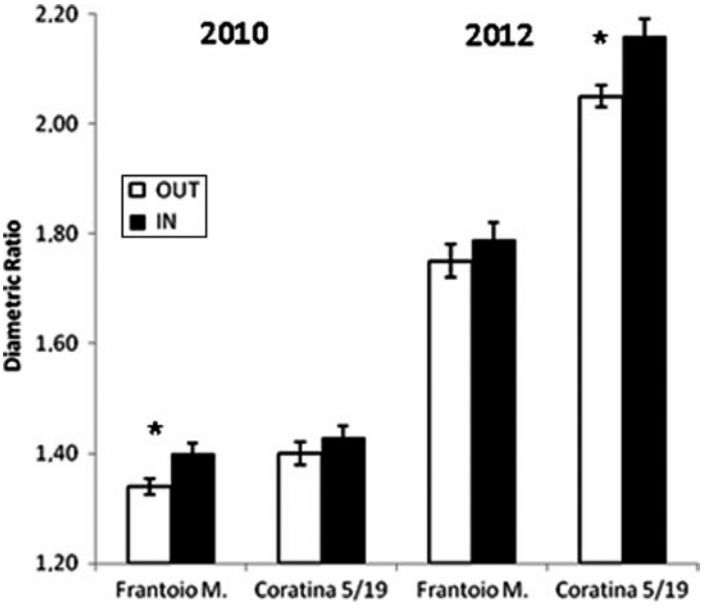

Figure 2. Diametric ratio of fruits detected at harvest time over two crop seasons (2010 and 2012) in samples from "out" and "in" canopy positions of "Frantoio M." and "Coratina 5/19" genotypes. The asterisk (*) denotes significant differences between "out" and "in" diametric ratio of fruits by Student's t-test ($p \leq 0.05$). Vales are mean ± SEM.

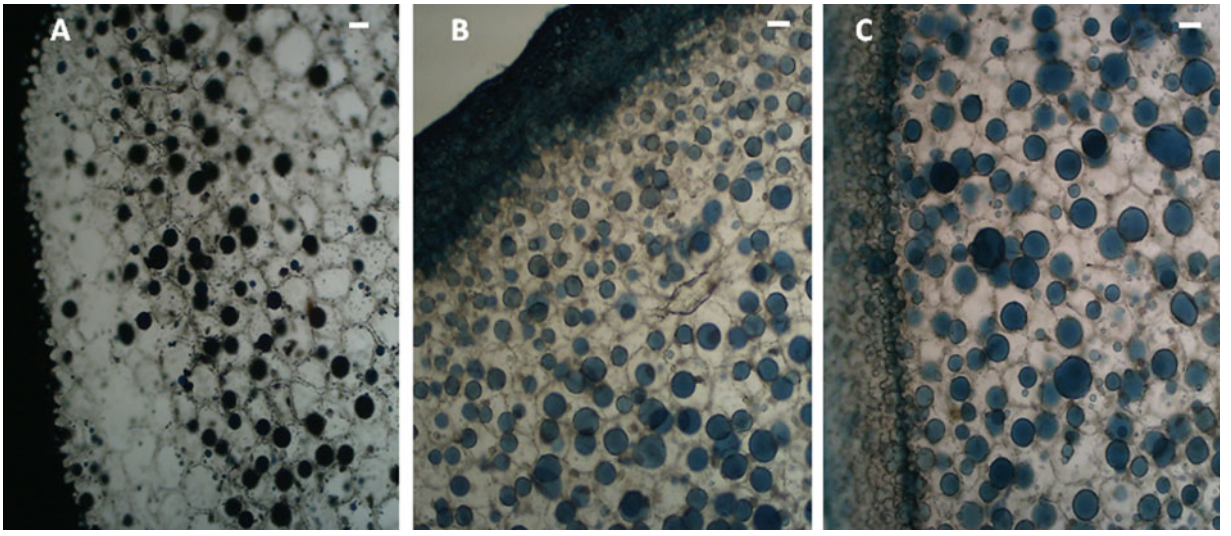

Figure 3. Vibratome sections of olive mesocarp of "Frantoio M." and "Coratina 5/19" genotypes observed under a light microscope (100 ×) after Sudan Black B staining. Oil bodies are blue–black stained at: (A) immature green fruit (50 DAFB); (B) greenish-yellow fruits (120 DAFB); and (C) mature fruit (160 DAFB). Scale bar: 50 μm.

similar trend in both cultivars (Figure 5). The enzyme activity increased from the first ripening phase (immature green fruit, 50 DAFB) to 120 DAFB when reached maximum values. During the late ripening phase (darkgoing stage, 160 DAFB), in 2010, the activity level markedly decreased, up to the same values observed at the immature green stage. On the other hand, in 2012, values decreased less drastically, maintaining relatively high levels, about 9 and 6 mmol min^{-1} g^{-1} in "Frantoio M." and "Coratina 5/19", respectively. Between the two cop seasons, "Frantoio M." showed noticeable changes in β-glucosidase activity, whereas in "Coratina 5/19" the enzyme activity was less changeable.

Concerning the β-glucosidase activity in relation to canopy fruit position, values found in both genotypes were significantly different only at 50 DAFB. At this time, "out" fruits showed a relatively higher enzyme level than "in" fruits. Successively, at 120 DAFB, an opposite state was observed and "out" fruits showed a loss of activity, ranging from 14% to 30%, depending on the genotype. Close to harvest time (160 DAFB), no marked differences between "in" and "out" fruits were observed.

Discussion

The reduced PAR level within canopy, reflecting on factors such as temperature and relative humidity, influenced the fruit growth determining some morphological and biochemical changes in comparison with fruits exposed to high irradiance. This feature was confirmed over two consecutive crop seasons characterized by different summer climatic conditions, mainly due to the water availability. The strong drought recorded in 2012 from June to August, when olive endocarp and mesocarp growth is particularly active according to the double-sigmoid curve growth pattern (Bollard 1970), determined a significant reduction in size and weight in both "in" and "out" fruits. The altered mesocarp cell division and expansion under water-deficit regimes were observed by several authors (Proietti & Antognozzi 1996; Costagli et al. 2003; Lavee et al. 2007; D'Andria et al. 2009). Moreover, this condition also determined a modification of fruit shape denoted by an increase in the diametric ratio in comparison with those typical of the two considered genotypes, as shown in Table I.

Shaded fruits developed at slow rate and, at harvest, they were of reduced size with a particular decrease in the pulp component. It is well known that natural and artificial shadings can determine an altered pathway of olive development (Proietti et al. 1994). A gradual reduced fruit daily growth rate, as a consequence of a decrease in the daily amount of phloem sap, determined a lack of carbohydrates necessary for fruit growth (Morandi et al. 2011; Yoon et al. 2011). Shading also slowed the darkgoing time of fruits that showed a tendency toward oblong shape as a strategy to intercept further irradiance, similarly to leaves that alter their morphology and anatomy as adaptation to low light levels (Gregoriou et al. 2007).

The reduced irradiance within canopy negatively influenced the lipid biosynthesis, determining a decreased number of oil bodies, mainly in "Coratina 5/19". It has been demonstrated that many yield components, including oil content, are limited by low solar radiation and need >50% of full sun PAR to obtain maximum values (Cherbiy-Hoffmann et al. 2012). On the other hand, the different PAR levels did not affect the oil body diameter that, during fruit

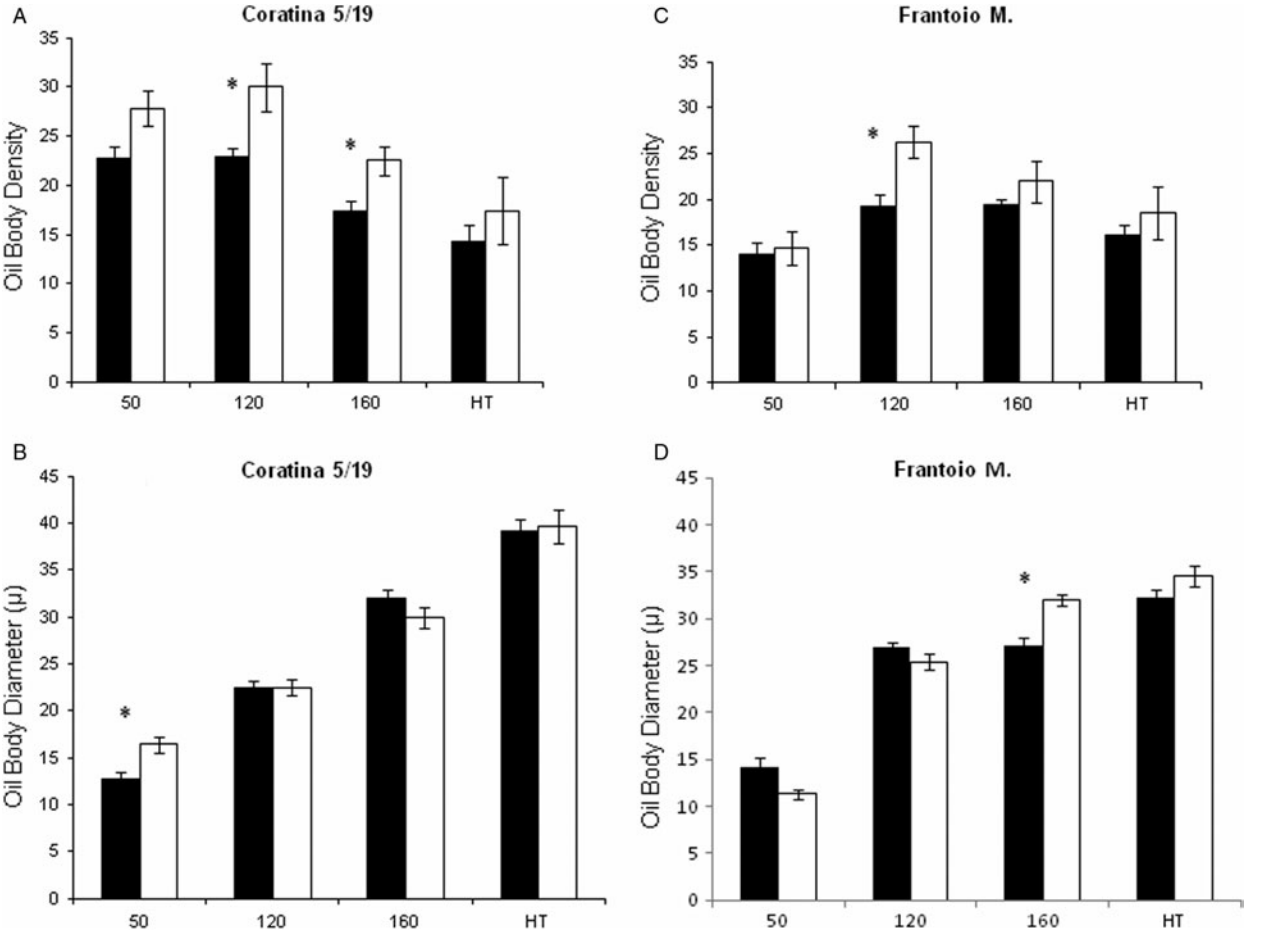

Figure 4. (A–D) Density ($n/50 \times 10^{-3}$ mm^2) and diameter (μ) of oil bodies in mesocarp cells of "Frantoio M." and "Coratina 5/19" olives sampled from "in" (■) and "out" (□) canopy positions at 50, 120, and 160 DAFB, and harvest time (HT). Values are means of two crop seasons ± SEM. The asterisk (*) denotes significant differences between "out" and "in" sample by Student's *t*-test ($p \leq 0.05$).

ripening, increased progressively, while a decrease in the oil body number occurred as a consequence of the coalescence phenomenon. The different climatic conditions, related to the dry summer of 2012, did not affect the oil body accumulation in mesocarp cells, in agreement with Breton et al. (2009) who suggested that oil accumulation is mostly independent of climatic variations.

A difference in the efficiency of oil accumulation between genotypes was observed. "Coratina 5/19", despite its extremely late darkgoing, had an early oil accumulation and, at immature green fruit, the oil body density was high and similar to that recorded at more advanced ripening stages. On the other hand, in "Frantoio M.", characterized by a gradual darkgoing, the beginning of lipid accumulation was delayed and the maximum oil body density was reached at the yellow–green stage. Thus, no relationship between ripening stage and oil accumulation time was found, as observed in other olive cultivars (Scamosci et al. 2011).

Concerning the β-glucosidase activity, no differences were found between the two analysed genotypes that were characterized by a similar trend during fruit ripening. The highest values were recorded at 120 DAFB, while, at more advanced ripening stage close to harvest, the enzyme activity decreased up to minimum levels measured in immature green fruits. These results agree with previous observations on different olive cultivars in which a β-glucosidase was identified whose activity trend during ripening appeared to be linked to oleuropein degradation (Briante et al. 2002; Mazzucca et al. 2006; Guitierrez-Rosales et al. 2010). The relatively high values recorded in fruits at harvest time, in the second crop season, could be related to the slowest ripening process, as a consequence of high temperature and very scarce rainfall that occurred during the summer. It is known that particular summer conditions, due to an increase in temperature, can reflect on longest phenological times (Moriondo & Bindi 2007). Moreover, drought conditions could have induced an accumulation of phenolic compounds that act as antioxidant defense (Llusià et al. 2014).

The enzyme analysis in "out" and "in" olives showed a different activity in relation to fruit-ripening

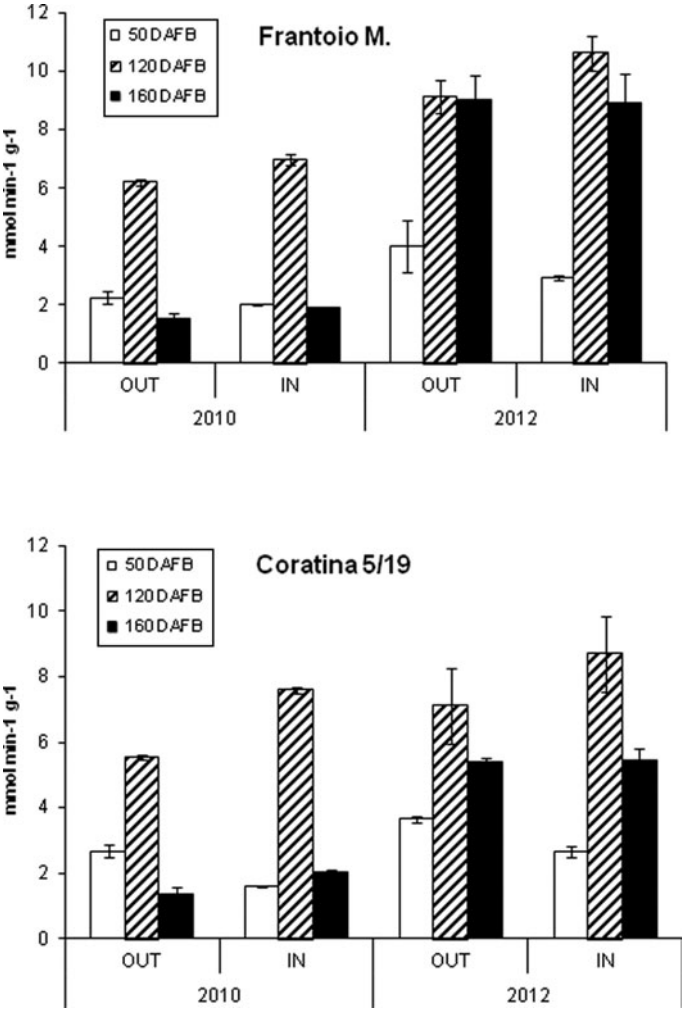

Figure 5. β-Glucosidase activity (mmol min^{-1} g^{-1}) in mesocarp tissue of "Frantoio M." and "Coratina 5/19" olives sampled from "in" and "out" canopy positions at 50, 120, and 160 DAFB, over two crop seasons (2010 and 2012). Values are mean ± SEM.

stage. At immature green stage (50 DAFB), enzyme values were greater in "out" fruits, while, at more advanced stages, the β-glucosidase activity was lower than "in" fruits. The critical role of β-glucosidase in shaping the phenolic profile of virgin oil was suggested by Romero-Segura et al. (2012) who found a positive correlation between enzyme activity and high phenols values. Taking into account this evidence, our results suggest to improve investigations about what might be a suitable sunlight exposure for the best metabolism conditions and phenolic compounds accumulation. It has been reported that environmental factors, such as excessive irradiation or high temperatures, can induce alterations and enzyme inactivation or enzyme structure modification (Léchaudel et al. 2013). The molecule α-glucosidase, sensitive to the photopolymerization, consistently showed a loss of activity when subjected to a direct polymerizing environment (Baroli et al. 2003). In grape, the influence of sunlight exposure on berry composition has been well documented, and an inhibition of phenolic synthesis under direct sunlight that matched with high temperature was found (Bergqvist et al. 2001; Lino et al. 2007).

Conclusions

This study confirms first that, regardless of the irradiance regime, rainfed summer conditions influenced negatively the morphometric characteristics of fruits and that the position of fruits in olive canopies may be a determinant factor for some physiological processes related to fruit growth and ripening. Shading inside the canopy, where the proportion of incident PAR intercepted by the crop was 47%, appeared to limit mostly the fruit growth and oil body accumulation in mesocarp cells. Some differences between the two considered genotypes were noted. In particular, "Coratina 5/19", despite its very late ripening time, was characterized by a fast fruit growth and early oil body accumulation, also under shading.

The canopy position influenced, in a different way, the β-glucosidase activity in relation to fruit-ripening stage. The lowest activity observed in "out" fruits could suggest a sensitivity of this enzyme to high PAR exposures that could be potentially negative. This latter issue requires further investigations considering that the levels of enzymatic activity in developing fruits may be important in determining the oil quality (Briante et al. 2002).

These findings indicate that providing an adequate and uniform lighting of the olive canopy, by careful choices of orchard management practices, can be a key factor for several yield components.

Acknowledgements

The authors acknowledge Mrs Sandra and Mr Silvano Pimpinelli for access to the olive orchards at their farm, Scarlino, Grosseto.

Funding

This research was partially supported by the Italian Ministry of University and Research (MIUR, PRIN 2008 "New technological approaches for the mechanical extraction of virgin olive oils and for wastewater treatments, addressed to the improvement of the quality and valorization of the wastewater microcomponents").

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