Irrigation and Fruit Canopy Position Modify Oil Quality of Olive Trees (cv. Frantoio)

Running Title: Water, light and olive oil quality

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Abstract

BACKGROUND: Fruit development and oil quality in *Olea europaea* L. are strongly influenced by both light and water availability. In this study the simultaneous effects of light environment and irrigation on fruit characteristics and oil quality were studied in a high-density orchard over two consecutive years. Olive fruits were harvested from three canopy positions (intercepting about 64%, 42% and 30% of above canopy radiation) of fully-productive trees subjected to full, deficit or complementary irrigation. RESULTS: Fruits receiving 61-67% of above canopy radiation showed the highest fruit weight, mesocarp oil content and maturation index, whereas those intercepting only 27-33% showed the lowest values. Palmitoleic and linoleic acids

increased in oils obtained from fruits exposed to high light levels, whereas oleic acid and the oleic-linoleic acid ratio decreased. Neither canopy position nor irrigation affected the K_{232}, K_{270}, ΔK, and lignans concentration in virgin olive oils (VOOs). Total phenols, 3,4-DHPEA-EDA and *p*-HPEA-EDA increased in VOOs produced from fruits harvested from the top of the canopy, whereas full irrigation decreased total phenols and 3,4-DHPEA-EDA concentrations with respect to the complementary irrigation treatment. CONCLUSION: Light and water availability are not only crucial for tree productivity, but they also clearly affect olive oil quality.

Keywords: fatty acids; mesocarp oil content; phenolic composition; photosynthetically active radiation; leaf water potential.

INTRODUCTION

Light and water are major driving forces for photosynthesis and transpiration, which are the main processes responsible for tree growth and productivity. In fruit trees and vines the evolution of planting systems from traditional orchards to high-density ones involved the optimization of canopy light interception and the introduction of irrigation [1, 2, 3]. High-density olive orchards became common since the 1980s, whereas very high-density (hedgerow) systems only became commercial since the middle of the 1990s [4]. As a result, studies on light interception and optimal management of irrigation in olive orchards are still relatively new despite the evidence about the marked effects that solar radiation and water have on vegetative activity, leaf morphology and density, photosynthesis, transpiration, fruit production and quality in fruit trees [3, 5, 6, 7, 8].

Connor [9] proposed a model that calculated profiles of photosynthetic active radiation (PAR) according to a combination of row height, width, spacing, and orientation of olive hedgerows, and associated these profiles with the productive responses of individual canopy walls. Trentacoste et al. [10] reported that olive fruits (cv. Arbequina) formed at the top of the canopy had higher fruit weight, mesocarp weight and oil content than fruits from less illuminated canopy zones. In a study conducted on 8-year-old olive trees (cv. Arbequina) planted at a 4 x 6 m tree spacing, Cherbiy-Hoffmann et al. [11] reported that fruit dry weight and oil content concentration increased linearly up to about 40% of external PAR if the light environment was manipulated

after endocarp sclerification. A similar relationship between canopy light interception and fruit oil content, albeit with a higher threshold (60% of incident PAR), had been determined earlier for hedgerow orchards [12]. In other experiments and simulations the fruit oil content increased linearly over the 12-75% interval of incident radiation, whereas fruit density increased linearly up to about 40% irradiance beyond which it remained stable [13]. As for the effect of canopy light interception on olive oil quality, Gómez-del-Campo and Garcia [14] reported that fruits located in the upper part of the canopies trained to hedgerows produced oils that were more stable, richer in polyphenols and saturated fatty acids than those obtained from less illuminated fruits. The current interest in light effects on oil quality of olive trees also stems from the progressive abandonment of severe pruning techniques, the adoption of free canopy training systems in high-density and hedgerow orchards, and the introduction of mechanical pruning.

Soil water availability virtually affects all aspects of tree performance including fruit development, fruit characteristics and oil quality [15, 16, 17]. Martinelli et al. [18] reported that fruits of rainfed olive trees had higher levels of total polyphenols than those collected from fully-irrigated trees during the period comprised between the post pit hardening stage and the complete pigmentation of the epicarp. At the last sampling date a higher concentration of anthocyanin in fruits sampled from rainfed trees was also observed, indicating that soil water availability affected ripening in olives [18]. In orchards and vineyards it has been shown that supplying water to fully compensate for plant water consumption does not necessarily lead to optimal fruit quality, and that periods of water deficit can improve quality depending on the timing of stress imposition [7, 19, 20, 21]. In mature olive trees extensive evidence has also been produced, showing that it is possible to reduce the amount of water applied during the irrigation season without negative effects on fruit and oil yield [22, 23, 24]. Caruso et al. [25] reported that the oil yield and the oil yield efficiency of deficit (46-54% of full irrigation) irrigated trees (cv. Frantoio) were 82 and 110% those of fully-irrigated ones over four years, respectively. The oil concentration in fruits of cv. Arbequina subjected to deficit irrigation (25% of the irrigation volume applied to the control treatment) was higher than that of fully-irrigated trees in two out of the three years of study [26]. Similar results were obtained in a hedgerow olive orchard (cv. Arbequina) where a reduction of irrigation by 70% in July allowed to

save 16% of total season irrigation water without losses in oil production compared with fully-irrigated trees [4].

Changes in the quality of VOO induced by soil water availability have been reported by many authors [16, 27, 28, 29]. Most studies showed a negative correlation between concentrations of phenols, ortho-diphenols, secoiridoids and the volume of water applied, whereas the irrigation regime had negligible effects on free acidity, peroxide value, fatty acid composition, and concentrations of lignans of VOOs [14, 16, 27, 29].

All the above reported studies focused on the effect of either water availability or light interception. In a previous work, we showed that there was an interaction between light intensity and tree water status on volatile organic compounds (VOCs) in VOOs, effects that could not be entirely predicted by simply summing the individual responses to light or water deficit [30]. The objective of this study was to determine the simultaneous effect of different light levels and tree water status on several parameters that characterize VOO quality. Free acidity, peroxide value, spectrophotometric indices, fatty acids composition, and phenolic compounds concentrations in VOOs obtained from trees grown in a high-density olive orchard were measured over two consecutive growing seasons.

EXPERIMENTAL

Plant material, radiation interception and tree water status

A high density (513 trees ha^{-1}) olive (*Olea europaea* L. cv. Frantoio) orchard, planted in April 2003 at the experimental farm of University of Pisa, Italy (43° 01'N; 10° 36' E), was used in 2008 and 2009. The soil was a sandy-loam permanently covered with a natural green cover. Canopies were trained to a free vase training system formed by a single trunk and three to five primary branches [25].

The climatic conditions over the study period were monitored using a weather station iMETOS IMT 300 (Pessl Instruments GmbH, Weiz, Austria) installed on site. Reference evapotranspiration, calculated according to the Penman–Monteith equation, was 993 and 1101 mm in 2008 and 2009, respectively. The effective evapotranspiration was calculated by using a crop coefficient (K_c) during the irrigation period of 0.55 and a coefficient of ground cover (K_r) of

0.9 and 1 in 2008 and 2009, respectively. Annual precipitation was 1107 and 771 mm in 2008 and 2009, respectively, while summer precipitation was 74 mm and 87 mm in those respective years. During the summer the average mean temperature was 23.1 and 23.3 °C in 2008 and 2009, respectively (Fig. 1). Annual solar radiation, measured by a silicon sensor placed on top of the weather station, was 71130 and 77501 W m^{-2} in 2008 and 2009, respectively. During the fruit development period (from anthesis through fruit harvest) solar radiation was 40935 and 46071 W m^{-2} in those two respective years, corresponding to 290 and 311 W m^{-2} of daily radiation. During the same period clear days (above canopy solar radiation to extra-terrestrial radiation greater than 0.75 [31]) were 66%, and cloudy days (above canopy solar radiation to extra-terrestrial radiation lower than 0.25 [31]) were 1% in 2008 [31]; in 2009 the percentage of sunny days and cloudy days was 72 and 2% respectively [31].

Subsurface drip irrigation was used to supply 100% (Full Irrigation, FI), 46-48% (Deficit Irrigation, DI) or 2-6% (Complementary Irrigation, CI) of tree water needs, calculated as effective evapotranspiration, for about 14 weeks. Irrigation periods were 2 July-10 October and 1 July-9 October in 2008 and 2009, respectively. Fully-irrigated trees received water 4-5 days a week and the volumes applied were 1860 and 2134 m^3 ha^{-1} in 2008 and 2009, respectively [25]. Fertilizers were applied via the sub-surface irrigation system every year before irrigation treatments were put into action. A total of 25 and 50 g of N, P_2O_5 and K_2O per tree were supplied to all trees in 2008 and 2009, respectively. The trees had started fruit production in 2005 (about 5 kg per tree) and had reached full production by the time the experiments were started [30].

Three trees per irrigation treatment were used in this experiment. In both years, on each of the selected trees, volumes of 1 m^3 each were identified in: i) the top zone of the canopy at a height of about 3 m (T); ii) the lower part of the South side at 2 m above ground (L-S); iii) the lower part of the North side at 2 m above ground (L-N). Each canopy position was replicated nine times (three trees per irrigation treatment for a total of nine trees).The PAR was measured at regular intervals from dawn until sunset on clear days with a LI-COR Line Quantum Sensor (LI-191 SB, Licor, Lincoln, USA) in 2008 and a Sun Scan System (SS1, Delta-T Devices Ltd, Cambridge, UK) in 2009. Two cross measurements of light interception (North-South and East-West

directions) were taken per each canopy position and the data averaged [30]. The average (2008-2009) canopy volume of FI, DI and CI trees was 27.9, 19.9 and 20.4 m^3, respectively.

Tree water status was determined by measuring the pre-dawn leaf water potential (PLWP) at 7-10 day intervals during the irrigation period using a pressure chamber [25]. In 2009 the stem water potential (SWP) was also determined (Fig. 3). The SWP was measured after blocking transpiration of leaves inserted near the main scaffolds of the tree [30]. The leaf was bagged and then sampled to determine SWP [32]. Preliminary measurements showed that the minimum time required for the leaf to reach equilibrium with the xylem was 40 min. In order to assess possible differences in leaf water potential due to different amounts of light intercepted, we also measured SWP on leaves located at different canopy positions of the same trees used for PLWP measurements. No differences in SWP between the different canopy positions were found (data not shown).

Fruit harvest, oil extraction and analysis

Full bloom occurred on 2 June and 24 May in 2008 and 2009, respectively and fruits were harvested on 21 and 19 October in those two respective years.

Due to the limited number of fruits in the 1 m^3 volume of selected canopy positions, we had to restrict the destructive sampling of fruits. Prior to harvest 50 fruits were sampled from each canopy position of each tree for the determination of fresh weight and maturation index. The oil content of the dry mesocarp of five fruits for each canopy position, previously sampled for fresh weight determinations, was measured by nuclear magnetic resonance (NMR) Oxford MQC-23 analyzer (Oxford Analytical Instruments Ltd., Oxford, UK), as previously reported [25].

Oil was extracted from about 1.5 kg of fruits sampled from each canopy position of each tree using an Abencor system (MC2, Ingenieria y Systemas, Sevilla, Spain) within 24 h from harvest [33]. From each sample we obtained about 100 mL of oil, that was then stored in the dark at 14 °C until analyses. Free acidity and peroxide value (PV) of oils were determined colorimetrically using an Oxitester unit (Olive Oxytester, CDR, Ginestra Fiorentina, FI, Italy) that allowed the determination of both parameters rapidly on small samples [33]. Fatty acid composition and UV

absorption characteristics at 232 and 270 nm of oils were measured in accordance with the European Official Methods [16, 34].

The fatty acid composition was determined in accordance with the European Official Methods [33, 35], peak identification of the various fatty acid methyl esters was performed by comparison of their retention times with those of Supelco 37 Component FAME Mix (Milan, Italy).

The phenolic composition was evaluated by liquid-liquid extraction from VOO and analyzed by high performance liquid chromatography (HPLC) [29, 35]. Standards were obtained from different sources: (3,4-dihydroxyphenyl)ethanol (3,4-DHPEA), produced by the Cayman Chemical Co. (Ann Arbor, MI, USA), was obtained from Cabru s.a.s. (Arcore, Milan, Italy), while the (*p*-hydroxyphenyl)ethanol (*p*-HPEA) was purchased from Fluka (Milan, Italy). The dialdehydic form of elenolic acid linked to 3,4-DHPEA or *p*-HPEA (3,4-DHPEA-EDA and *p*-HPEA-EDA, respectively, the isomer of oleuropein aglycon (3,4-DHPEA-EA), the (+)-1-acetoxypinoresinol, and (+)-pinoresinol were extracted from VOO and separated by semipreparative HPLC according to previously reported procedures [29, 36].

Experimental design and statistical analysis

A one factor randomized complete block design was used with canopy position as the fixed factor and irrigation level as the randomized factor. Three trees per irrigation treatment (a total of nine trees) were selected similar in size, productivity and location within the orchard. Means of irrigation treatments and canopy positions were separated by least significant differences (LSD) at $p \leq 0.05$ after analysis of variance using MSTAT software (Michigan State University, East Lansing, USA). Fatty acids composition data were subjected to ANOVA after arcsine transformation. Where applicable, data were analyzed by regression using Costat (CoHort Software, Monterey, USA).

Fatty acid composition and phenolic concentrations of VOOs were subjected to Principal Components Analysis (PCA) using the SIMCA 13.0 chemometric package (Umetrics AB, Umeå, Sweden). The raw data were normalized by subtracting the mean, and autoscaled by dividing them by the standard deviation. The number of significant components was found by cross-validation, and the results of PCA modeling were presented in graphical form.

RESULTS

Climatic and experimental conditions during fruit development were similar in both growing seasons (Fig. 1), except that summer precipitations were higher in 2009 than in 2008. The amount of the average daily PAR intercepted at each canopy position was similar in 2008 and 2009. Diurnal profiles of light interception showed that PAR values were 67, 46 and 33% of above canopy ones for T, L-S and L-N positions, respectively in 2008 and 61, 38 and 27% in 2009 (Fig. 2).

The PLWP of the FI trees was usually maintained above –1.0 MPa with an average of –0.86 MPa. The PLWP of DI trees decreased progressively with increasing seasonal drought and reached -2.2 and -2.6 MPa at 76 and 110 days after full bloom (DAFB) in 2008 and 2009, respectively (Fig. 3A, B). The minimum PLWP values of CI trees were -3.5 MPa in 2008 (109 DAFB) and -4.8 MPa in 2009 (102 DAFB). In 2008 and 2009 the PLWP of both DI and CI treatments rose to values similar to FI twice because of rainfall during the irrigation period (Fig. 3A, B). In the 2009 seasonal course of SWP of fully-irrigated trees (PLWP of about -0.9 MPa) was similar to that of PLWP and ranged between -1.3 and -1.9 MPa (Fig. 3 C).

Fully irrigated, deficit irrigated and complementary irrigated trees produced 18.869 ± 4.303, 14.232 ± 0.940 and 11.192 ± 1.285 kg of fruit per tree in 2008, respectively, and 23.130 ± 5.286, 10.931 ± 1.023 and 8.430 ± 1.273 kg per tree in 2009 (values are means ± standard error of three trees for each irrigation treatment).

Both canopy position and water status affected fruit fresh weight (FW), but the effect of location was more evident than that of irrigation: the fruit FW from the T canopy position was 131-140% that from the L-N, whereas the fruit FW from the FI treatment was 119-114% that of CI fruits (Fig. 4). Maturation was also markedly affected by both canopy position and irrigation (Fig. 4C, D). Fruits harvested from the top of the canopy of CI trees showed highest maturation index. Low light levels significantly slowed down the development of dark colour and the progression of fruit maturation. The oil content in the mesocarp increased at an apparently steady rate as the level of light interception increased up to a threshold level of about 40% PAR, beyond which it

levelled off (Fig. 4E, F). In 2008 fruit mesocarps from the top of the canopy had 105% of the oil (% dry weight) of the L-N and L-S ones, (107 and 102% respectively, in 2009).

Peroxide value of VOOs produced in 2008 showed significant differences due to different tree water status and light interception (Tab. 1). In 2008 maximum peroxide values, all below the limits of VOO classification (EU Off. J. Eur. Communities, 2003), were measured in oils from fruits of the top part of the canopy of FI trees, whereas minimum values were obtained in oils from the L-N part of the canopy of CI trees that received only occasional irrigations (Tab. 1). Oils obtained from fruits located in the L-S position had intermediate values. A significant interaction between the irrigation regime and canopy position was found for peroxide value in 2008, but not in 2009. In 2009 those parameters were similar regardless of tree water status or canopy position (Tab. 1). In 2009 all spectrophotometric indices were unaffected by either light or water regime, except for the ΔK that was significantly affected by light interception (Tab. 1).

Oil fatty acid composition was more influenced by the canopy position than the irrigation regime (Tab. 2), without any significant interaction between the two factors (supplementary material_Table 1). Oleic acid decreased as light interception increased and showed the highest values in oils obtained from fruits that intercepted the lowest amount of light (Tab. 2). Significant differences in oleic acid concentration between T and L-N zones were observed in both years (Tab. 2). Linolenic acid decreased as light levels increased only in 2009; linoleic and palmitoleic acids increased as light interception increased, and showed significant differences between oils from T and L-N zones in both years (Tab. 2). At high PAR levels the oleic-linoleic ratio of VOOs was lower than that of fruits exposed to low PAR values in both years. However, canopy position did not affect the saturated-unsaturated fatty acids ratio. Stearic acid was significantly lower in FI than in CI trees in both years (Tab. 2).

Both tree water status and canopy position influenced the phenolic concentrations of VOOs. In general, trees that had undergone the least water deficit or fruits in the L-N position produced oils with the lowest concentration of phenolic compounds. The interaction between I x CP was never significant for any of the different fractions, except for *p*-HPEA in 2008 (supplementary material _Table 2). The *p*-HPEA showed in both years a decrease in oils obtained from trees that experienced high level of water stress. Oils obtained from fruits in the top layer of the

canopy had higher concentrations of *p*-HPEA-EDA (183-223%), *p*-HPEA (116-173%), 3,4-DHPEA-EDA (178-238%), and the sum of phenolic compounds (155-159%) than those from the Low-North side in both years (Tab. 3). However, only the *p*-HPEA-EDA showed significant differences between canopy zones in both years, whereas the other phenolic compounds and their sum showed significant differences only in one of the two years (Tab. 3). There was a positive correlation between 3,4-DHPEA-EDA, *p*-HPEA-EDA, the total phenolic concentration and the amount of intercepted radiation in both years (data not shown).

The model obtained from PCA explained 74% of the total variance (46%, 15%, and 13% for the first, the second and the third component, respectively). The score plot of the second component vs. the first one showed a clear discrimination of the objects according to light exposure in the first component, while the second one evidenced their discrimination based on water status (Fig. 9). The third component referred to the year effect, which was the least evident of the three components (data not shown). From the relative loading plot it turns out that the variables responsible for the differentiation of the objects in the first component were mainly oleic and linolenic acids that reached the highest values for the VOOs obtained from olives from L-N canopy position; on the other hand, the palmitoleic and linoleic acids and the phenolic compounds were in the right side of this component with the highest levels in VOOs produced from the top of the canopy (Fig. 9). Regarding the second component the variables with the highest absolute loading values were stearic acid, arachidic acid (Top) and *p*-HPEA (bottom). The highest values for stearic and arachidic acids were measured in VOOs obtained from complementary irrigated trees, whereas the higher concentrations of *p*-HPEA were observed under full irrigation conditions.

DISCUSSION

Previous studies on the effect of either the light environment or the water status on VOO quality did not consider what happened when both factors were changed concomitantly. In a recent article we showed that the interaction between irrigation, canopy position, and year significantly affected the development of fruit colour change (and thus the progression of maturation) and the concentration of many volatile compounds in VOO [30]. In the current study we confirmed

individual effects of tree water status and light exposure, but we did not find any evidence of significant interactions between those factors on spectrophotometric indices, fatty acid composition, phenolic composition and concentration of VOO. The only interaction appeared for free acidity and peroxide value in the first year of study, but not in the second one.

Irrigation and light exposure affected fruit characteristics in both years. Fruit fresh weight increased with higher PLWP (less water deficit), and fruit colour change was delayed when trees were fully irrigated. It is well known that better water status increases fruit weight and size of several olive cultivars and that fruit weight of fully-irrigated trees can be higher than that of deficit irrigated trees [4, 25]. Gucci et al. [22] reported that fruit fresh weight of fully- and deficit-irrigated trees was 158% and 123% (average of two years), respectively, of that from non-irrigated ones. Fresh weight and maturation index increased almost linearly within the range of measured light levels. Fruits growing in well exposed parts of the canopy were usually larger and heavier than those in less illuminated zones [12, 37]. In our study, the effect of light on mesocarp oil content was quite similar to that reported for cv. Arbequina in the Southern hemisphere [11]. The mesocarp oil content increased almost linearly until a level of about 40% PAR beyond which it levelled off, similarly to the threshold value reported by Cherbiy-Hoffman et al. [11], but lower than the 60% value of horizontally intercepted radiation reported for hedgerows orchards by Connor et al. [12]. Our average fruit density for fully-irrigated trees were 740 fruits m^{-3} of canopy volume, corresponding to a medium-high crop load [11, 38]. The average fruit density was low for the DI and CI treatments (320 and 261 fruits m^{-3} of canopy volume, respectively).

Besides the above described effects on fruit characteristics, both light and irrigation markedly affected VOO quality. In 2009 ΔK was significantly affected by light interception, whereas K_{232} and K_{270} were unaffected by either light environment or water regime. On the contrary, Gómez-del-Campo and Garcia [14] measured an increase of the K_{232} and K_{270} as fruits were sampled from upper canopy layers. In another study Proietti et al. [39] reported that free acidity and peroxide value were unaffected by light conditions. Apparently conflicting results also emerged about the effect of water availability, confirming previously published reports [29, 40]. In the first year of our study higher values of peroxide were measured in oils obtained from FI trees, but differences disappeared in the second year. Other authors observed differences in peroxide values of oils

316 obtained from trees subjected to different irrigation treatments but these results were not
317 consistent between the two years of study [41, 42]. Variability due to the growing season and
318 processing conditions may be responsible for the increase in free acidity and peroxide value of
319 VOOs from irrigated treatments. This issue needs to be further addressed at the biochemical
320 level to clarify which substrates and enzymatic reactions in fruit metabolism may be modified by
321 irrigation.
322 The effect of light intercepted by fruits on fatty acid composition of VOOs was consistent in both
323 years: as light interception increased oleic acid decreased, whereas palmitoleic and linoleic
324 acids increased with significant differences between VOOs obtained from the Top and the Low-
325 North parts of the canopy. Our results are in agreement with those reported for hedgerows
326 orchards of cv. Arbequina by Gómez-del-Campo and Garcia [14], who measured significantly
327 higher values of palmitic, palmitoleic and linoleic acids in the upper canopy layers. Although fruit
328 temperature was not measured in either study, it is reasonable to expect higher temperature in
329 canopy zones that are more exposed to solar radiation. If so, temperature might explain the
330 observed changes in fatty acid composition, since it had been previously shown that the oleic
331 acid concentrations decreased linearly with increasing temperature in the 16-32 °C range,
332 whereas palmitoleic, linoleic and linolenic acids increased [42]. Similar PLWP values in the
333 different zones of the canopy seem to exclude that leaf water relations were responsible for the
334 changes in the fatty acid composition.
335 Tree water status affected the phenolic concentration of VOOs, consistently with existing
336 literature [16, 28, 29]. The slightly different response between the two years may have been due to
337 summer rains summer in the second year that increased data variability. In both years VOOs
338 from trees with higher water status did show lower phenolic concentrations. In both years fruits
339 harvested from the top layer of the canopy produced oils with higher concentrations of total
340 phenols, *p*-HPEA-EDA, *p*-HPEA, and 3,4-DHPEA-EDA than those obtained from the less
341 exposed ones (Low-North side). Oils produced from the Low-South part showed intermediate
342 values between Top and Low-North ones. Interestingly, high phenolic concentrations were
343 measured in VOOs obtained from fruits at a more advanced stage of maturation, assessed
344 visually as tissue pigmentation. This is usually not the case since more ripe olives reportedly

yield oils with lower phenolic concentrations [44]. However, in agreement with our results, Gómez-del-Campo and Garcia [14] reported that VOOs extracted from fruits located in the upper layers of the canopy had significantly higher contents of *p*-HPEA-EDA, 3,4-DHPEA-EA, ortho-diphenols, secoiridoids derivatives and total phenols. In another study conducted on cvs. Frantoio and Leccino, Proietti et al. [39] observed that fruits grown under high light conditions produced oils with a higher polyphenol content and better sensorial characteristics than those obtained from shaded fruits. Hence, good exposure to light stimulates phenolic accumulation in the fruit and the oil. In particular, both the 3,4-DHPEA-EDA and the *p*-HPEA-EDA decreased at low light levels. The response of 3,4-DHPEA and 3,4-DHPEA-EA to light was less clear as it varied between the two years of study.

In conclusion, we showed that both the light environment and water availability modified VOO quality. The PCA model confirmed that the discrimination effect of canopy position was greater than that of water status in both years. The effect of the year was the least evident, contrarily to what had been previously reported for VOCs [30]. Tree water status mainly influenced fruit size, fruit pigmentation and phenolic concentration in the oil, whereas light exposure also affected fatty acids composition. The interaction between canopy position and irrigation on VOO parameters was seldom significant. The study has important implications on correct orchard management for maximum VOOs quality. Light interception can be manipulated by selecting row orientation, planting distance and training system. These factors do not only play a role in determining performance and productivity of trees, but also clearly affect oil quality. Analogously, water availability can be optimized by appropriate site selection and deficit irrigation management to produce top quality VOOs.

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Table 1. Free acidity (g of oleic acid/100 g), peroxide value (meq O_2 kg^{-1}), K_{232}, K_{270} and ΔK of VOOs obtained from olive fruits sampled from three different canopy positions (top, L-S and L-N) of trees (cv. Frantoio) grown under different irrigation regimes (full, deficit and complementary) in 2008 and 2009

	2008		2009				
	Free acidity	Peroxide value	Free acidity	Peroxide value	K_{232}	K_{270}	ΔK
Irrigation							
Full	0.1	5.1 a	0.11	8.0	1.97	0.12	−0.0005
Deficit	0.1	5.0 a	0.09	8.1	2.02	0.15	−0.0012
Complementary	0.1	3.0 b	0.07	7.9	2.04	0.14	−0.0009
Canopy position							
Top	0.1	5.2 a	0.09	7.8	2.03	0.15	−0.0009 ab
L-S	0.1	4.2 ab	0.09	7.7	2.02	0.14	−0.0005 a
L-N	0.1	3.7 b	0.09	8.5	1.97	0.12	−0.0013 b
Source of variation							
I	NS	0.0001	NS	NS	NS	NS	NS
CP	NS	0.034	NS	NS	NS	NS	0.048
I × CP	NS	0.043	NS	NS	NS	NS	NS

Values are means of nine VOO samples. Different lowercase letters indicate least significant differences between tree water status or canopy position after ANOVA within each year ($P \leq 0.05$). I, irrigation; CP, canopy position; NS, not significant. $^{*}P < 0.0001$.

Table 2. Fatty acid composition (%) of VOOs obtained from olive fruits sampled from three different canopy positions (top, L-S and L-N) of trees (cv. Frantoio) grown under different irrigation regimes (full, deficit and complementary) in 2008 and 2009

		Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic	Arachidic	Oleic/linoleic	Saturated/ unsaturated
Year	Irrigation									
2008	Full	11.2	1.1	2.0 b	76.7	7.4	0.7	0.4	10.6	0.2
	Deficit	10.9	1.2	2.0 b	76.1	8.2	0.7	0.4	9.3	0.2
	Complementary	11.0	1.2	2.5 a	76.0	7.8	0.7	0.4	9.9	0.2
	Canopy position									
	Top	11.4	1.3 a	2.2 a	74.9 b	8.6 a	0.7	0.4	8.8 c	0.2
	L-S	11.0	1.2 a	2.2 a	76.3 ab	7.8 b	0.7	0.4	9.9 b	0.2
	L-N	10.7	1.0 b	2.1 b	77.5 a	7.0 c	0.7	0.4	11.2 a	0.2
2009	Irrigation									
	Full	14.2 b	1.4	2.0 b	71.9	9.3	0.7	0.4	7.9	0.2
	Deficit	14.8 a	1.3	2.2 ab	71.8	8.8	0.6	0.4	8.4	0.2
	Complementary	14.0 b	1.3	2.4 a	71.8	9.2	0.7	0.4	8.0	0.2
	Canopy position									
	Top	14.1	1.5a	2.2	71.2 b	9.8 a	0.6 b	0.4	7.4 b	0.2
	L-S	14.3	1.4a	2.2	71.4 b	9.7 a	0.6 b	0.4	7.4 b	0.2
	L-N	14.7	1.1b	2.1	73.0 a	7.7 b	0.7 a	0.4	9.5 a	0.2

Values are means of nine VOO samples. Different lowercse letters indicate least significant differences between tree water status or canopy position after ANOVA ($P \leq 0.05$). Data were subjected to ANOVA after arcsine transformation. I, irrigation; CP, canopy position.

Table 3. Total phenols and phenolic composition (mg kg^{-1}) of VOOs obtained from olive fruits sampled from three different canopy positions (top, L-S and L-N) of trees (cv. Frantoio) grown under different irrigation regimes (full, deficit and complementary) in 2008 and 2009

		3,4-DHPEA	*p*-HPEA	3,4-DHPEA-EDA	*p*-HPEA-EDA	3,4-DHPEA-EA	(+)-1-acetoxipinoresinol	(+)-pinoresinol	∑ of phenolic compounds
Year	Irrigation								
2008	Full	4.0	6.8 a	180 b	58	139 b	28 a	24	440 c
	Deficit	3.5	6.8 a	206 a	76	149 b	31 a	25	496 b
	Complementary	3.2	4.4 b	223 a	76	204 a	22 b	21	554 a
	Canopy position								
	Top	3.7	7.8 a	252	98 a	180	27	24	593
	L-S	4.2	5.7 b	215	69 b	170	27	24	514
	L-N	2.8	4.5 b	142	44 c	141	27	22	382
2009	Irrigation (I)								
	Full	3.3	9.2	208	72 b	158	35	28	515
	Deficit	4.8	8.1	178	100 ab	192	32	26	541
	Complementary	1.9	5.7	210	130 a	160	32	26	566
	Canopy position								
	Top	4.0	8.1	267 a	126 a	164 b	34	30	633 a
	L-S	2.8	7.9	217 a	107 a	198 a	31	27	591 a
	L-N	3.1	7.0	112 b	69 b	149 b	34	24	398 b

Values are means of nine VOO samples. Different lowercase letters indicate least significant differences between tree water status or canopy position after analysis of variance ($P \leq 0.05$). I, irrigation; CP, canopy position.

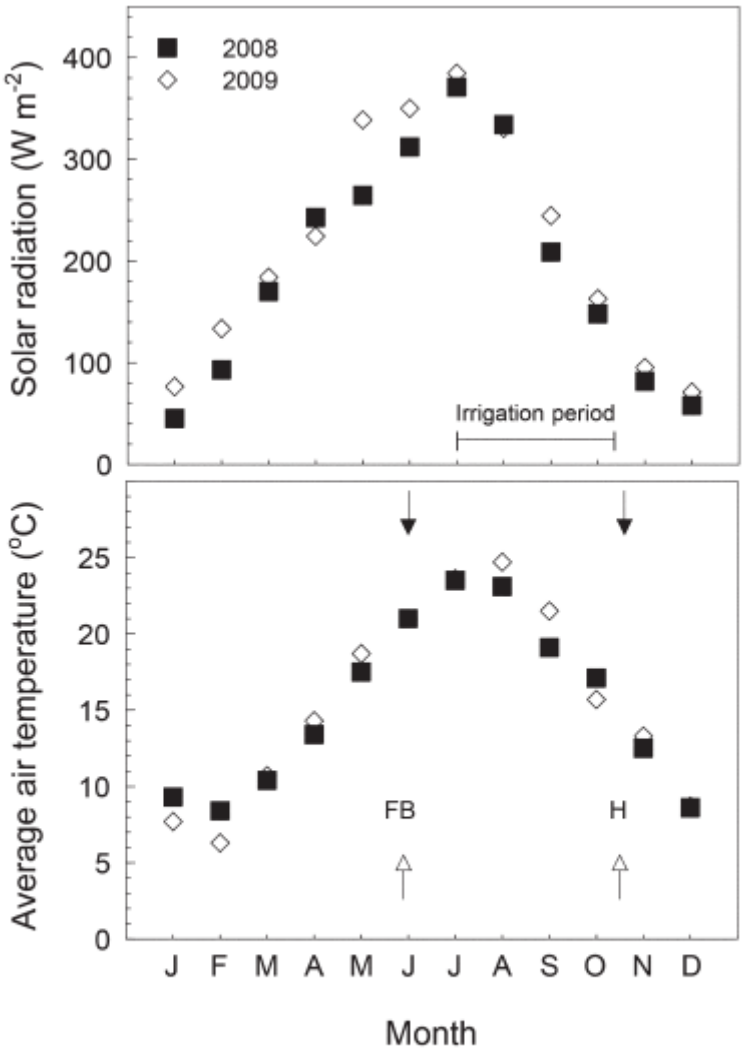

Figure 1. Mean monthly values of solar radiation and average air temperature at the experimental site in 2008 (closed symbols) and in 2009 (open symbols). Arrows indicate the dates of full bloom (FB) and harvest (H) in those respective years; the horizontal line indicates the irrigation period in both years.

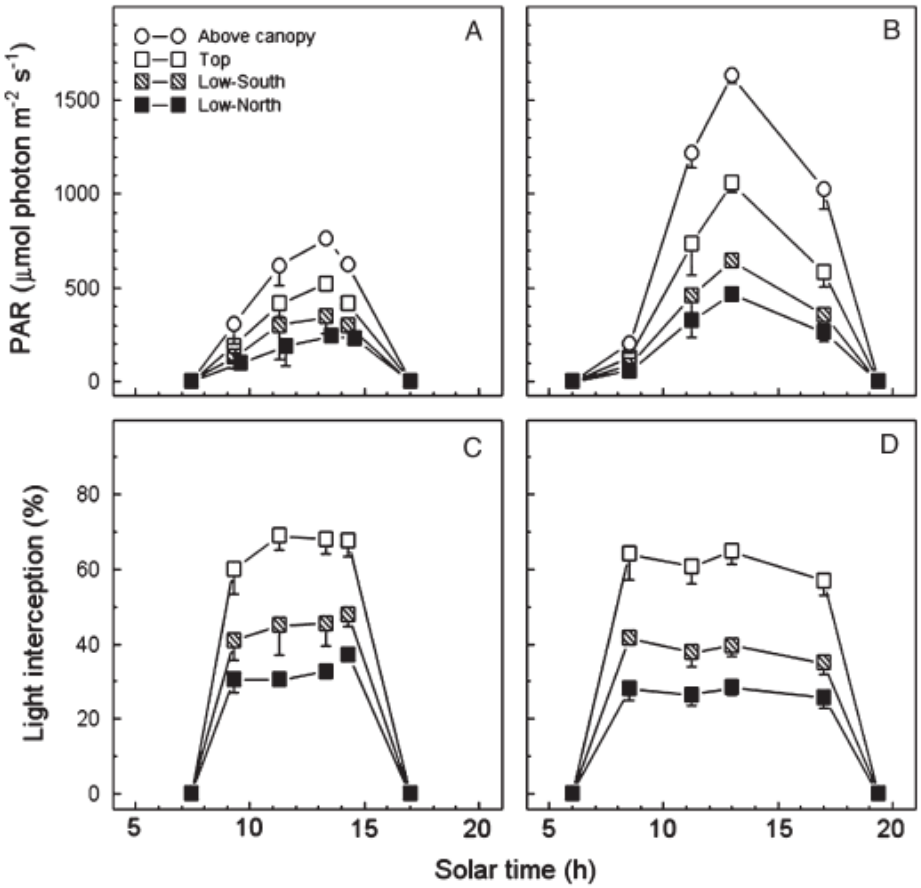

Figure 2. Diurnal courses of PAR (A, B) and light interception, expressed as percentage of above canopy measurements, (C, D) Measured at th positions (top, L-S and L-N) of olive trees grown in the field under full, deficit or complementary irrigation regimes in December 2008 (C) and 2009 (D). Symbols are mean-SD of nine replicates.

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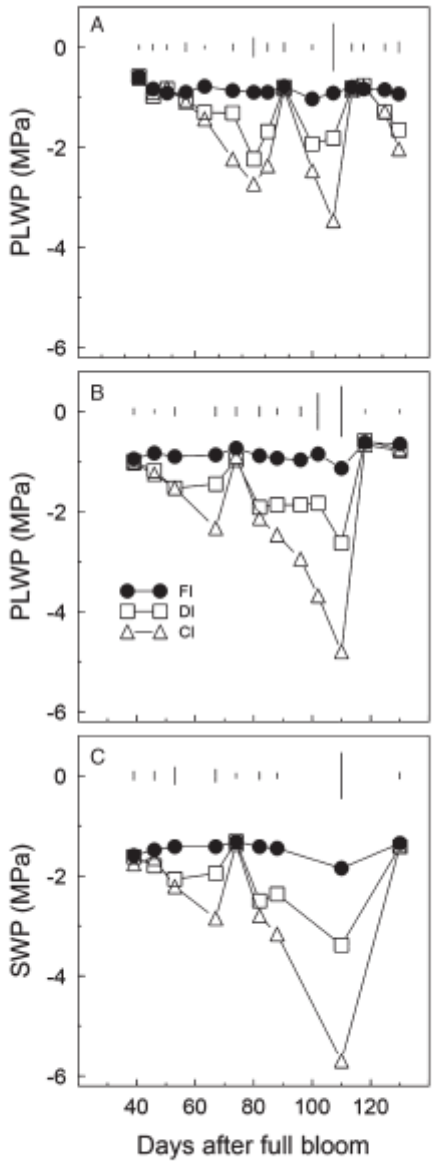

Figure 3. Seasonal course of PLWP and SWP of olive trees grown in the field under full (FI), deficit (DI) or complementary (CI) irrigation in 2008 (A) and 2009 (B, C). Symbols are the means of three trees. Vertical bars represent least significant differences ($P < 0.05$) calculated after analysis of variance within each date of measurement.

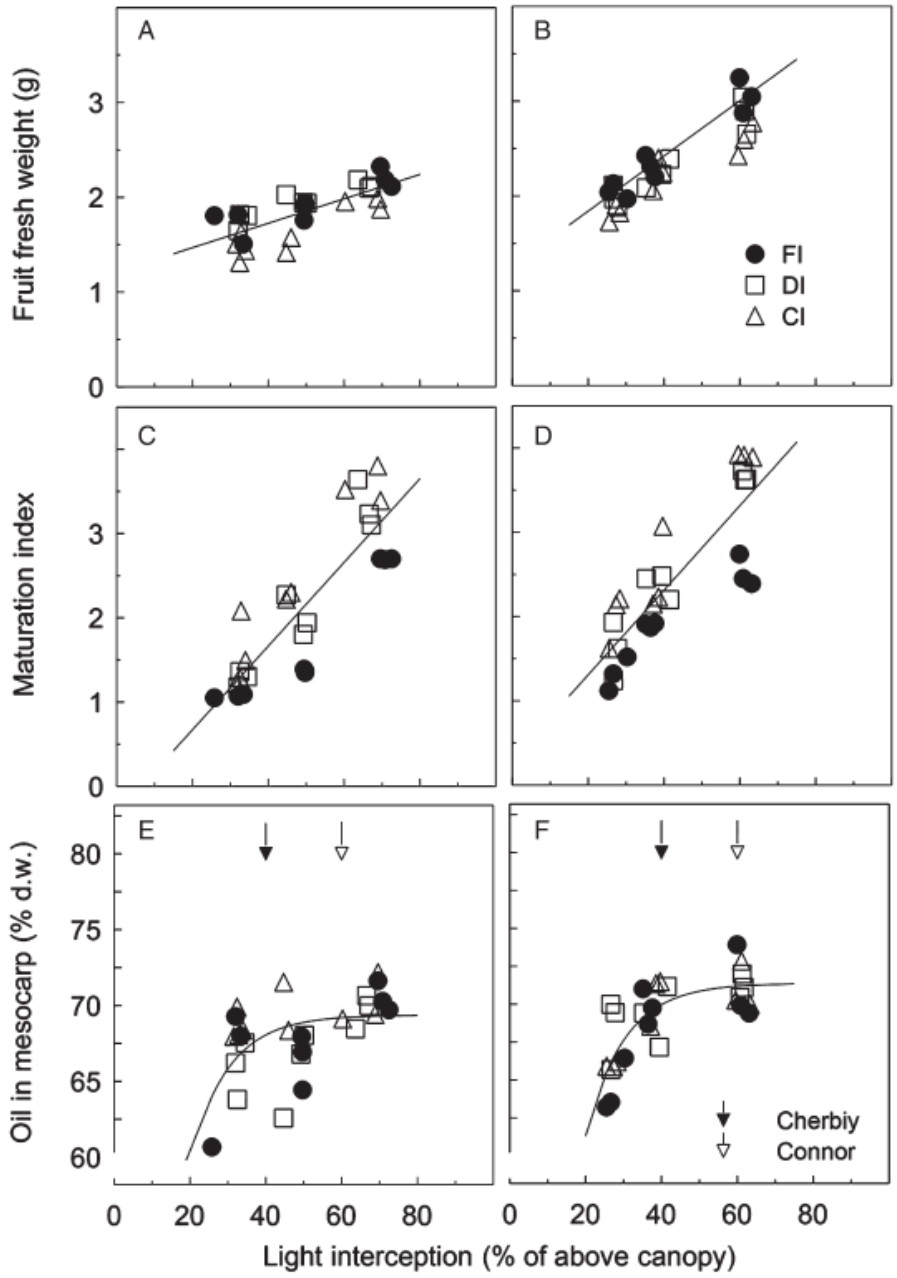

Figure 4. The relationship between fruit fresh weight (A, B), maturation index (C, D), mesocarp oil content (E, F) and light interception of olive fruits sampl from three different canopy positions of trees grown in the field under full (FI), deficit (DI) or complementary (CI) irrigation in 2008 (A, C, E) and 2009 (B, D, Different symbols indicate trees with different water status. Values of fresh weight are the means of 50 fruits per canopy position, and those of oil conte are of five replicates. Regression equations: (A) $y = 0.013x + 1.21$, $r^2 = 0.57$***; (B) $y = 0.026x + 1.27$, $r^2 = 0.82$***; (C) $y = 0.05x + 0.32$, $r^2 = 0.73$***; ($y = 0.05x + 0.30$, $r^2 = 0.72$***; (E) $y = 69.4\ (1 - e^{-0.099x})$, $r^2 = 0.28$**; (F) $y = 71.4\ (1 - e^{-0.098x})$, $r^2 = 0.56$***. Arrows indicate the light interception thresho beyond which the fruit oil content did not increase linearly in previous studies by Cherbiy-Hoffmann *et al.*[11] (closed symbol) and Connor *et al.*[12] (op symbol), respectively.

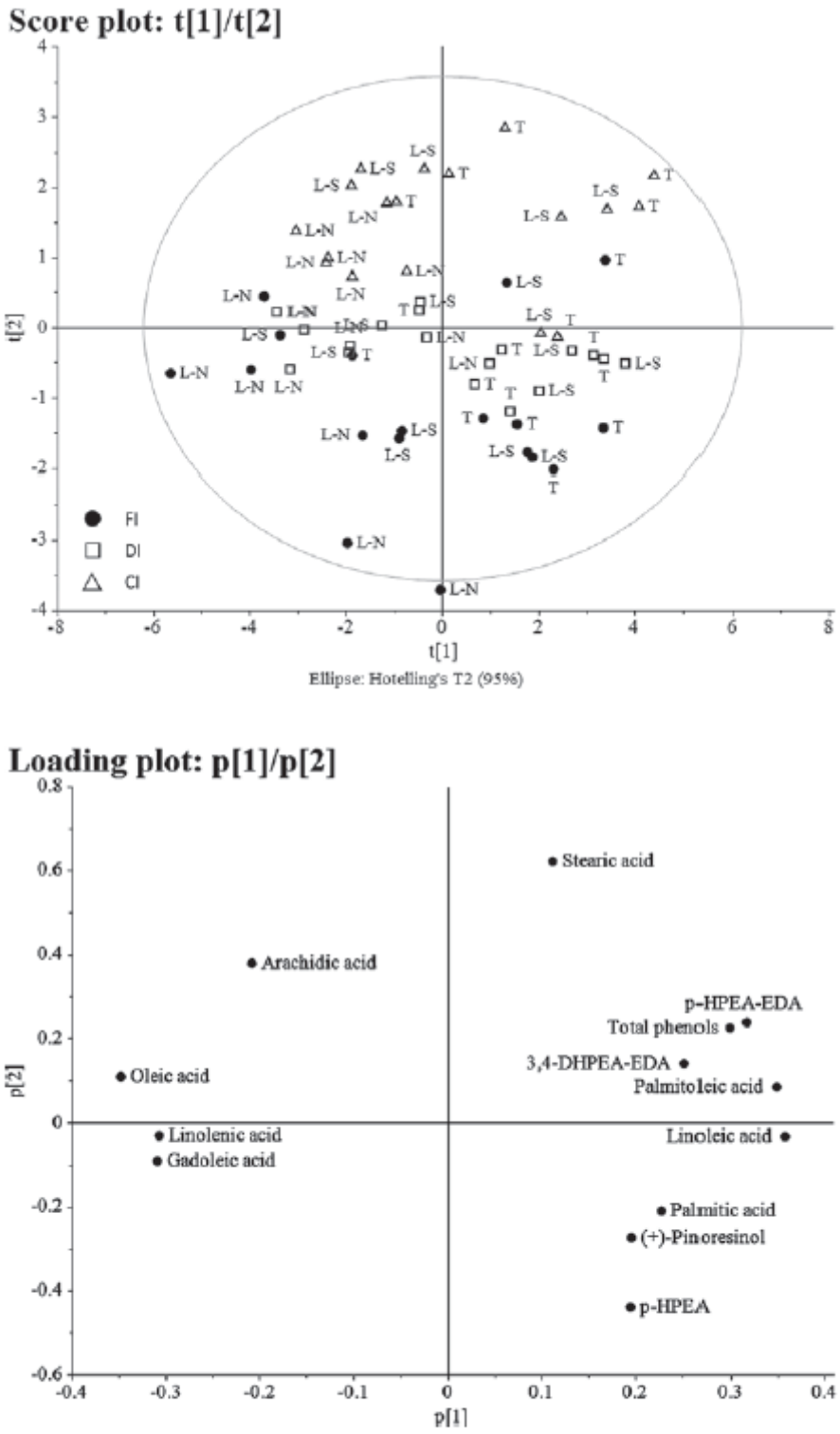

Figure 5. Score plot and loading plot of the first two principal components of the PCA model built with all the virgin olive oils obtained in the 2 years using fatty acids and phenolic composition as variables. Different symbols in the score plot indicate trees grown in the field under full (FI), deficit (DI) or complementary (CI) irrigation. Each symbol represents one olive oil sample. T, top.

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