

## 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

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

37  
38 **Keywords:** fatty acids; mesocarp oil content; phenolic composition; photosynthetically active  
39 radiation; leaf water potential.

## 41 INTRODUCTION

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

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

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

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

87 save 16% of total season irrigation water without losses in oil production compared with fully-  
88 irrigated trees <sup>4</sup>.

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

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

103

## 104 **EXPERIMENTAL**

### 105 **Plant material, radiation interception and tree water status**

106 A high density (513 trees ha<sup>-1</sup>) olive (*Olea europaea* L. cv. Frantoio) orchard, planted in April  
107 2003 at the experimental farm of University of Pisa, Italy (43° 01'N; 10° 36' E), was used in 2008  
108 and 2009. The soil was a sandy-loam permanently covered with a natural green cover. Canopies  
109 were trained to a free vase training system formed by a single trunk and three to five primary  
110 branches <sup>25</sup>.

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

116 0.9 and 1 in 2008 and 2009, respectively. Annual precipitation was 1107 and 771 mm in 2008  
117 and 2009, respectively, while summer precipitation was 74 mm and 87 mm in those respective  
118 years. During the summer the average mean temperature was 23.1 and 23.3 °C in 2008 and  
119 2009, respectively (Fig. 1). Annual solar radiation, measured by a silicon sensor placed on top of  
120 the weather station, was 71130 and 77501 W m<sup>-2</sup> in 2008 and 2009, respectively. During the fruit  
121 development period (from anthesis through fruit harvest) solar radiation was 40935 and 46071 W  
122 m<sup>-2</sup> in those two respective years, corresponding to 290 and 311 W m<sup>-2</sup> of daily radiation. During  
123 the same period clear days (above canopy solar radiation to extra-terrestrial radiation greater  
124 than 0.75<sup>31</sup>) were 66%, and cloudy days (above canopy solar radiation to extra-terrestrial  
125 radiation lower than 0.25<sup>31</sup>) were 1% in 2008<sup>31</sup>; in 2009 the percentage of sunny days and  
126 cloudy days was 72 and 2% respectively<sup>31</sup>.

127 Subsurface drip irrigation was used to supply 100% (Full Irrigation, FI), 46-48% (Deficit Irrigation,  
128 DI) or 2-6% (Complementary Irrigation, CI) of tree water needs, calculated as effective  
129 evapotranspiration, for about 14 weeks. Irrigation periods were 2 July-10 October and 1 July-9  
130 October in 2008 and 2009, respectively. Fully-irrigated trees received water 4-5 days a week and  
131 the volumes applied were 1860 and 2134 m<sup>3</sup> ha<sup>-1</sup> in 2008 and 2009, respectively<sup>25</sup>. Fertilizers  
132 were applied via the sub-surface irrigation system every year before irrigation treatments were  
133 put into action. A total of 25 and 50 g of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O per tree were supplied to all trees in  
134 2008 and 2009, respectively. The trees had started fruit production in 2005 (about 5 kg per tree)  
135 and had reached full production by the time the experiments were started<sup>30</sup>.

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

144 directions) were taken per each canopy position and the data averaged <sup>30</sup>. The average (2008-  
145 2009) canopy volume of FI, DI and CI trees was 27.9, 19.9 and 20.4 m<sup>3</sup>, respectively.  
146 Tree water status was determined by measuring the pre-dawn leaf water potential (PLWP) at 7-  
147 10 day intervals during the irrigation period using a pressure chamber <sup>25</sup>. In 2009 the stem water  
148 potential (SWP) was also determined (Fig. 3). The SWP was measured after blocking  
149 transpiration of leaves inserted near the main scaffolds of the tree <sup>30</sup>. The leaf was bagged and  
150 then sampled to determine SWP <sup>32</sup>. Preliminary measurements showed that the minimum time  
151 required for the leaf to reach equilibrium with the xylem was 40 min. In order to assess possible  
152 differences in leaf water potential due to different amounts of light intercepted, we also measured  
153 SWP on leaves located at different canopy positions of the same trees used for PLWP  
154 measurements. No differences in SWP between the different canopy positions were found (data  
155 not shown).

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#### 157 **Fruit harvest, oil extraction and analysis**

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

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

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

172 absorption characteristics at 232 and 270 nm of oils were measured in accordance with the  
173 European Official Methods <sup>16, 34</sup>.

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

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

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### 187 **Experimental design and statistical analysis**

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

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

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## RESULTS

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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).

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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).

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

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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

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

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

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

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

258 canopy had higher concentrations of *p*-HPEA-EDA (183-223%), *p*-HPEA (116-173%), 3,4-  
259 DHPEA-EDA (178-238%), and the sum of phenolic compounds (155-159%) than those from the  
260 Low-North side in both years (Tab. 3). However, only the *p*-HPEA-EDA showed significant  
261 differences between canopy zones in both years, whereas the other phenolic compounds and  
262 their sum showed significant differences only in one of the two years (Tab. 3). There was a  
263 positive correlation between 3,4-DHPEA-EDA, *p*-HPEA-EDA, the total phenolic concentration  
264 and the amount of intercepted radiation in both years (data not shown).  
265 The model obtained from PCA explained 74% of the total variance (46%, 15%, and 13% for the  
266 first, the second and the third component, respectively). The score plot of the second component  
267 vs. the first one showed a clear discrimination of the objects according to light exposure in the  
268 first component, while the second one evidenced their discrimination based on water status (Fig.  
269 9). The third component referred to the year effect, which was the least evident of the three  
270 components (data not shown). From the relative loading plot it turns out that the variables  
271 responsible for the differentiation of the objects in the first component were mainly oleic and  
272 linolenic acids that reached the highest values for the VOOs obtained from olives from L-N  
273 canopy position; on the other hand, the palmitoleic and linoleic acids and the phenolic  
274 compounds were in the right side of this component with the highest levels in VOOs produced  
275 from the top of the canopy (Fig. 9). Regarding the second component the variables with the  
276 highest absolute loading values were stearic acid, arachidic acid (Top) and *p*-HPEA (bottom).  
277 The highest values for stearic and arachidic acids were measured in VOOs obtained from  
278 complementary irrigated trees, whereas the higher concentrations of *p*-HPEA were observed  
279 under full irrigation conditions.

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## DISCUSSION

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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<sup>30</sup>. In the current study we confirmed

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

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

307 Besides the above described effects on fruit characteristics, both light and irrigation markedly  
308 affected VOO quality. In 2009  $\Delta K$  was significantly affected by light interception, whereas  $K_{232}$   
309 and  $K_{270}$  were unaffected by either light environment or water regime. On the contrary, Gómez-  
310 del-Campo and Garcia <sup>14</sup> measured an increase of the  $K_{232}$  and  $K_{270}$  as fruits were sampled from  
311 upper canopy layers. In another study Proietti et al. <sup>39</sup> reported that free acidity and peroxide  
312 value were unaffected by light conditions. Apparently conflicting results also emerged about the  
313 effect of water availability, confirming previously published reports <sup>29, 40</sup>. In the first year of our  
314 study higher values of peroxide were measured in oils obtained from FI trees, but differences  
315 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 <sup>41, 42</sup>. 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 <sup>14</sup>, 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 <sup>42</sup>. 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 <sup>16, 28, 29</sup>. 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

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

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

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## REFERENCES

369

- 370 1. Jackson JE, Light interception and utilization by orchard systems *Horticultural Reviews* **2**: 208-  
371 267 (1980).
- 372 2. Palmer JW, Canopy manipulation for optimal utilization of light In *Manipulation of fruiting* C J  
Wright Ed London: Butterwoths 245-262 (1989).

- 373 3. Fereres E, Evans RG, Irrigation of fruit trees and vines: an introduction *Irrig Sci* **24**: 55-57  
374 (2006).
- 375 4. Gómez-del-Campo M, Summer deficit-irrigation strategies in a hedgerow olive orchard cv  
376 'Arbequina': effect on fruit characteristics and yield *Irrig Sci* **31**: 259-269 (2013).
- 377 5. Palmer JW, Jackson JE, Seasonal light interception and canopy development in hedgerow  
378 and bed system apple orchards *J App Ecol* **14**: 539-549 (1977).
- 379 6. Doud DS, Ferree DC, Influence of altered light levels on growth and fruiting of mature  
380 "Delicious" apple trees *J Am Soc Hortic Sci* **105**: 325-328 (1980).
- 381 7. Behboudian MH, Mills TM, Deficit irrigation in deciduous orchards *Hortic Rev* **21**: 105-131  
382 (1997).
- 383 8. Gregoriou K, Pontikis K, Vemmos S, Effects of reduced irradiance on leaf morphology  
384 photosynthetic capacity and fruit yield in olive (*Olea europaea* L) *Photosynthetica* **45**: 172-181  
385 (2007).
- 386 9. Connor DJ, Towards optimal designs for hedgerow olive orchards *Austr J Agr Res* **57**: 1067-  
387 1072 (2006).
- 388 10. Trentacoste ER, Gómez-del-Campo M, Rapoport HF, Olive fruit growth tissue development  
389 and composition as affected by irradiance received in different hedgerow positions and  
390 orientations *Sci Hortic* **198**: 284-293 (2016).
- 391 11. Cherbiy-Hoffmann SU, Hall AJ, Rousseaux MC, Fruit yield and vegetative growth responses to  
392 photosynthetically active radiation during oil synthesis in olive trees *Sci Hortic* **150**: 110-116  
393 (2013).
- 394 12. Connor DJ, Centeno A, Gómez-del-Campo M, Yield determination in olive hedgerow orchards II  
395 Analysis of radiation and fruiting profiles *Crop and Pasture Science* **60**: 443-452 (2009).
- 396 13. Connor DJ, Gómez-del-Campo M, Trentacoste ER, Relationships between olive yield  
397 components and simulated irradiance within hedgerows of various row orientations and  
398 spacings *Sci Hortic* **198**: 12-20 (2016).
- 399 14. Gómez-del-Campo M, Garcia JM, Canopy fruit location can affect olive oil quality in  
400 'Arbequina' hedgerow orchards *J Am Oil Chem Soc* **89**: 129-133 (2012).

- 401 15. Rapoport HF, Costagli G, Gucci R, The effect of water deficit during early fruit development on  
402 olive fruit morphogenesis *J Am Soc Hortic Sci* **129**: 121-127 (2004).
- 403 16. Servili M, Esposito S, Lodolini EM, Selvaggini R Taticchi A, Urbani S, Montedoro GF,  
404 Serravalle M, Gucci R, Irrigation effects on quality phenolic composition and selected volatiles  
405 of virgin olive oil cv Leccino *J Agric Food Chem* **55**: 6609-6618 (2007).
- 406 17. Gucci R, Lodolini EM, Rapoport HF, Water deficit-induced changes in mesocarp cellular  
407 processes and the relationship between mesocarp and endocarp during olive fruit  
408 development *Tree Physiol* **29**: 1575-1585 (2009).
- 409 18. Martinelli F, Basile B, Morelli G, d'Andria R, Tonutti P, Effects of irrigation on fruit ripening  
410 behaviour and metabolic changes in olive *Sci Hortic* **144**: 201-207 (2012).
- 411 19. Matthews MA, Anderson MM, Fruit ripening in *Vitis vinifera* L: responses to seasonal water  
412 deficits *Am J Enol Vitic* **39**: 313-320 (1988).
- 413 20. Marsal J, Lopez G, del Campo J, Mata M, Arbones A, Girona J, Postharvest regulated deficit  
414 irrigation in 'Summit' sweet cherry: fruit yield and quality in the following season *Irrig Sci* **28**:  
415 181-189 (2010).
- 416 21. Fereres E, Goldhamer DA, Sadras V, Smith M, Marsal J, Girona J, Naor A, Gucci R, Caliendo  
417 A, Ruz C, Yield response to water of fruit trees and vines: guidelines In *Crop yield response to*  
418 *water* P Steduto TC Hsiao E Fereres & D Raes (Eds) Rome: FAO 2012 Vol 66 pp 246-295.
- 419 22. Moriana A, Orgaz F, Pastor M, Fereres E, Yield responses of a mature olive orchard to water  
420 deficits *J Am Soc Hort Sci* **123**: 425-431 (2003).
- 421 23. Gucci R, Lodolini E, Rapoport HF, Productivity of olive tree with different water status and crop  
422 load *J Hort Sci Biotech* **82**: 648-656 (2007).
- 423 24. Lavee S, Hanoch E, Wodner M, Abramowitch E, The effect of predetermined deficit irrigation  
424 on the performance of cv Muhasan olives (*Olea europaea* L) in the eastern coastal plain of  
425 Israel *Sci Hortic* **99**: 267-277 (2007).
- 426 25. Caruso G, Rapoport HF, Gucci R, Long-term evaluation of yield components of young olive  
427 trees during the onset of fruit production under different irrigation regimes *Irrig Sci* **31**: 37-47  
428 (2013).

- 429 26. Iniesta F, Testi L, Orgaz F, Villalobos FJ, The effects of regulated and continuous deficit  
430 irrigation on the water use growth and yield of olive trees *Eur J Agron* **30**: 258-265 (2009).
- 431 27. Tovar MJ, Motilva MJ, Romero MP, Changes in the phenolic composition of virgin olive oil  
432 from young trees (*Olea europaea* L cv Arbequina) grown under linear irrigation strategies *J*  
433 *Agric Food Chem* **49**: 5502-5508 (2001).
- 434 28. Gómez-Rico A, Salvador MD, Moriana A, Pérez D, Olmedilla N, Ribas F, Fregapane G,  
435 Influence of different irrigation strategies in a traditional Cornicabra cv olive orchard on virgin  
436 olive oil composition and quality *Food Chem* **100**: 568-578 (2007).
- 437 29. Caruso G, Gucci R, Urbani S, Esposto S, Taticchi A, Di Maio I, Selvaggini R, Servili M, Effect  
438 of different irrigation volumes during fruit development on quality of virgin olive oil of cv  
439 Frantoio *Agric Water Manage* **134**: 94-103 (2014).
- 440 30. Benelli G, Caruso G, Giunti G, Cuzzola A, Saba A, Raffaelli A, Gucci R, Changes in olive oil  
441 VOCs induced by water status and light environment in canopies of *Olea europaea* L trees *J*  
442 *Sci Food Agr* **95**: 2473-2481 (2015).
- 443 31. Allen RG, Pereira LS, Raes D, M Smith In *Crop evapotranspiration-Guidelines for computing*  
444 *crop water requirements-FAO Irrigation and drainage paper 56* (Eds) Rome: FAO 1998 Vol  
445 300 (9) p 300.
- 446 32. Begg JE, Turner NC Water potential gradients in field tobacco *Plant Physiol* **46**: 343-346  
447 (1970).
- 448 33. Gucci R, Caruso G, Canale A, Loni A, Raspi A, Urbani S, Taticchi A, Esposto S, Servili M,  
449 Qualitative changes of olive oils from fruits damaged by *Bactrocera oleae* (Rossi). *HortScience*  
450 **47(2)**: 301-306 (2012).
- 451 34. EU Off J Eur Communities 6 November 2003 Regulation 1989/03 amending Regulation (EEC).  
452 No 2568/91 on the characteristics of olive oil and olive-pomace oil and on the relevant  
453 methods of analysis modifies the EEC No 2568/91 on olive oils and pomace olive oils  
454 characteristics and relative analysis methods Official Journal L 295/57 13/11/2003.
- 455 35. Selvaggini R, Servili M, Urbani S, Esposto S, Taticchi A, Montedoro GF, Evaluation of phenolic  
456 compounds in virgin olive oil by direct injection in high-performance liquid chromatography with  
457 fluorimetric detection *J Agric Food Chem* **54**: 2832-2838 (2006).



- 458 36. Montedoro GF, Servili M, Baldioli M, Selvaggini R, Miniati E, Macchioni A, Simple and  
459 hydrolyzable compounds in virgin olive oil 3 Spectroscopic characterization of the secoridoids  
460 derivatives *J Agric Food Chem* **41**: 2228-2234 (1993).
- 461 37. Bartolini S, Leccese A, Andreini L, Influence of canopy fruit location on morphological  
462 histochemical and biochemical changes in two oil olive cultivars *Plant Biosystems* **148**: 1221-  
463 1230 (2014).
- 464 38. Trentacoste ER, Puertas CM, Sadras VO, Effect of fruit load on oil yield components and  
465 dynamics of fruit growth and oil accumulation in olive (*Olea europaea* L). *Eur J Agron* **32**: 249-  
466 254 (2010).
- 467 39. Proietti P, Nasini L, Famiani F, Guelfi P, Standardi A, Influence of light availability in fruit and  
468 oil characteristics in *Olea europaea* L *Acta Hort* **949**: 243-249 (2012).
- 469 40. Dag A, Ben Gal A, Yermiyahu U, Basheer L, Nir Y, Zohar K, The effect of irrigation level and  
470 harvest mechanization on virgin olive oil quality in a traditional rain-fed 'Souri' olive orchard  
471 converted to irrigation *J Sci Food Agric* **88**: 1524-1528 (2008).
- 472 41. Berenguer MJ, Vossen PM, Grattan SR, Connell JH, Polito VS, Tree irrigation level for  
473 optimum chemical and sensory properties of olive oil *HortScience* **41**: 427-432 (2006).
- 474 42. Stefanoudaki E, Williams M, Chartzoulakis K, Harwood J, Effect of irrigation on quality  
475 attributes of olive oil *J Agric Food Chem* **57**: 7048-7055 (2009).
- 476 43. García-Inza GP, Castro DN, Hall AJ, Rousseaux MC, Responses to temperature of fruit dry  
477 weight oil concentration and oil fatty acid composition in olive (*Olea europaea* L var 'Arauco').  
478 *Eur J Agron* **54**: 107-115 (2014).
- 479 44. Gómez-Rico A, Salvador MD, La Greca M, Fregapane G, Phenolic and volatile compounds of  
480 extra virgin olive oil (*Olea europaea* L Cv Cornicabra) with regard to fruit ripening and irrigation  
481 management *J Agric Food Chem* **54**: 7130-7136 (2006).

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**Table 1.** Free acidity (g of oleic acid/100 g), peroxide value (meq O<sub>2</sub> kg<sup>-1</sup>), K<sub>232</sub>, K<sub>270</sub> 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 <sub>232</sub>	K <sub>270</sub>	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 lowercase 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.

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**Table 3.** Total phenols and phenolic composition ( $\text{mg kg}^{-1}$ ) 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	<i>p</i> -HPEA	3,4-DHPEA-EDA	<i>p</i> -HPEA-EDA	3,4-DHPEA-EA	(+)-1-acetoxipinoresinol	(+)-pinoresinol	$\Sigma$ 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.

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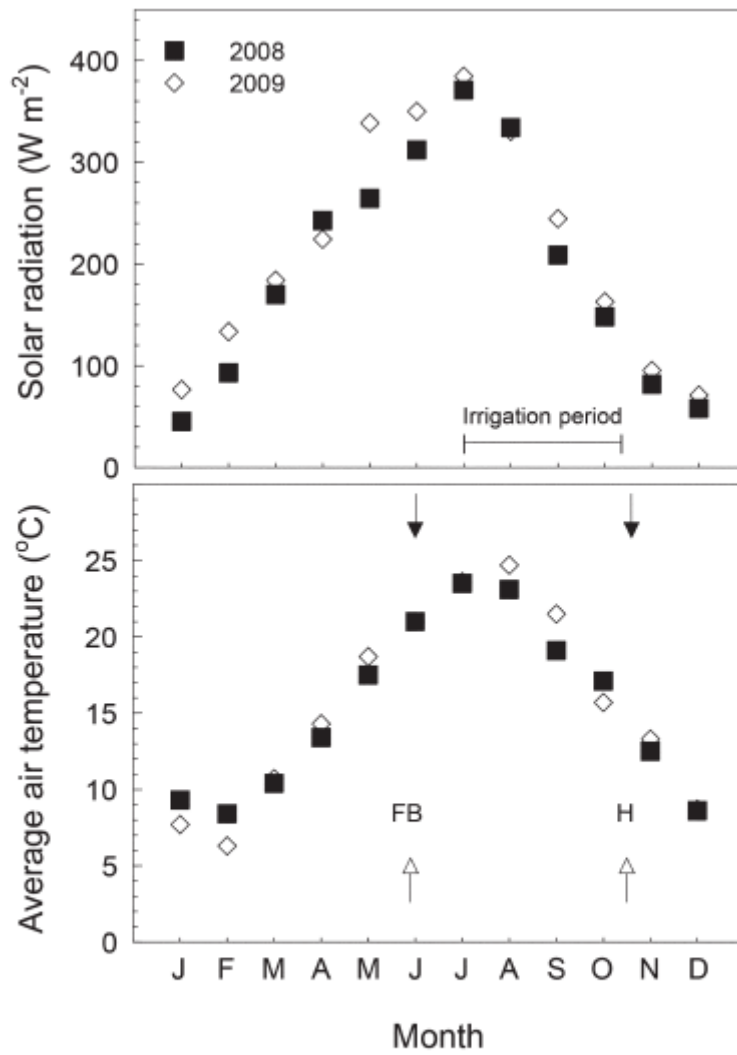
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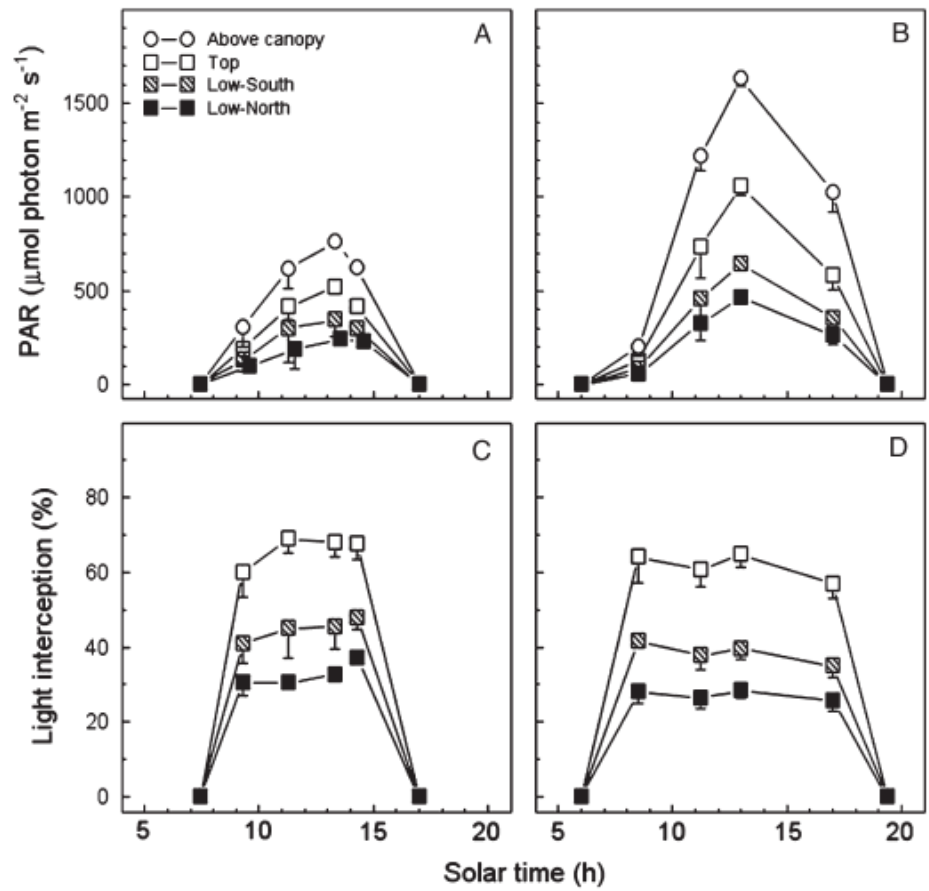
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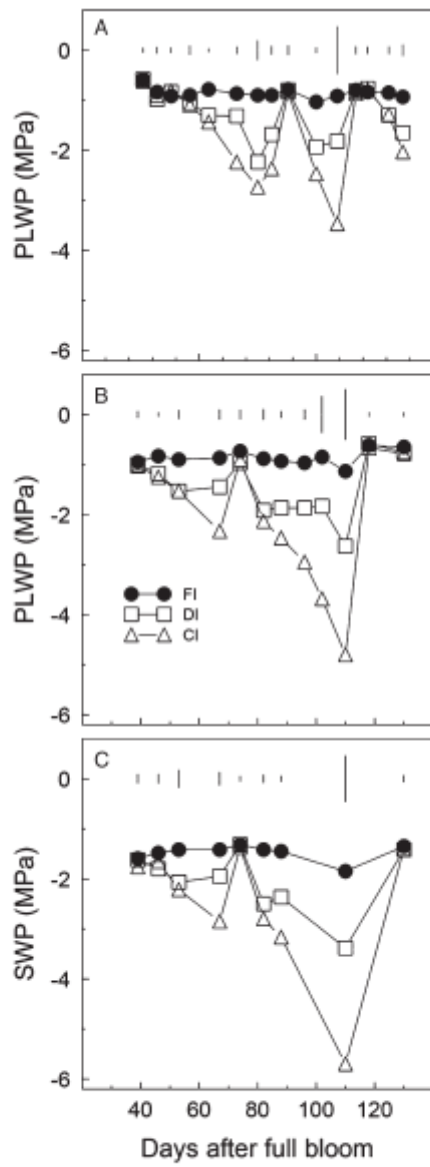
**Figure 1.** Mean monthly values of solar radiation and average air temperature at the experimental site in 2008 (closed symbols) and in 2009 (open symbols). Arrows indicate the dates of full bloom (FB) and harvest (H) in those respective years; the horizontal line indicates the irrigation period in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

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**Figure 2.** Diurnal courses of PAR (A, B) and light interception, expressed as percentage of above canopy measurements, (C, D) Measured at the 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.

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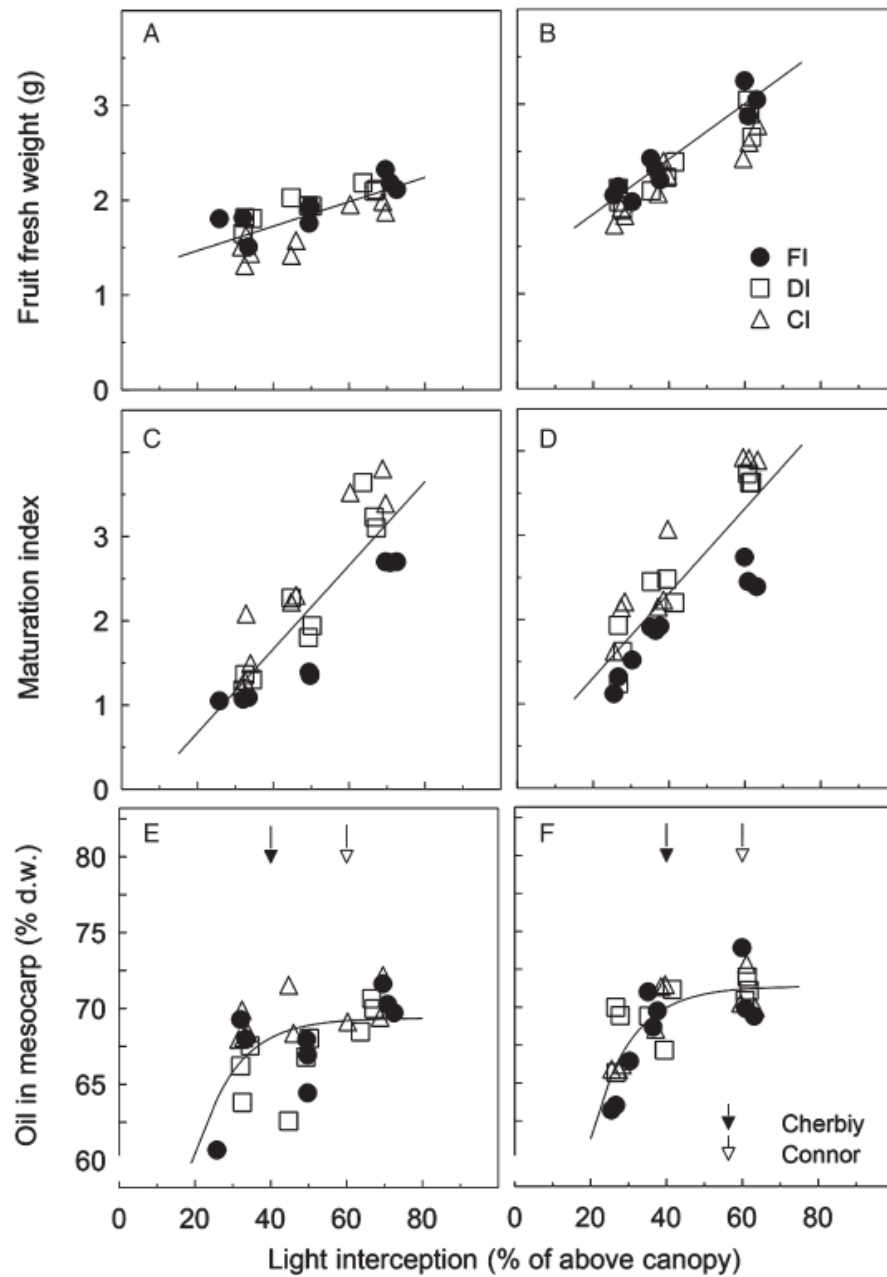
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**Figure 4.** The relationship between fruit fresh weight (A, B), maturation index (C, D), mesocarp oil content (E, F) and light interception of olive fruits sampled from three different canopy positions of trees grown in the field under full (FI), 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 content 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)  $y = 0.05x + 0.32$ ,  $r^2 = 0.73^{***}$ ; (D)  $y = 0.05x + 0.30$ ,  $r^2 = 0.72^{***}$ ; (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 threshold beyond which the fruit oil content did not increase linearly in previous studies by Cherbiy-Hoffmann *et al.*<sup>11</sup> (closed symbol) and Connor *et al.*<sup>12</sup> (open symbol), respectively.

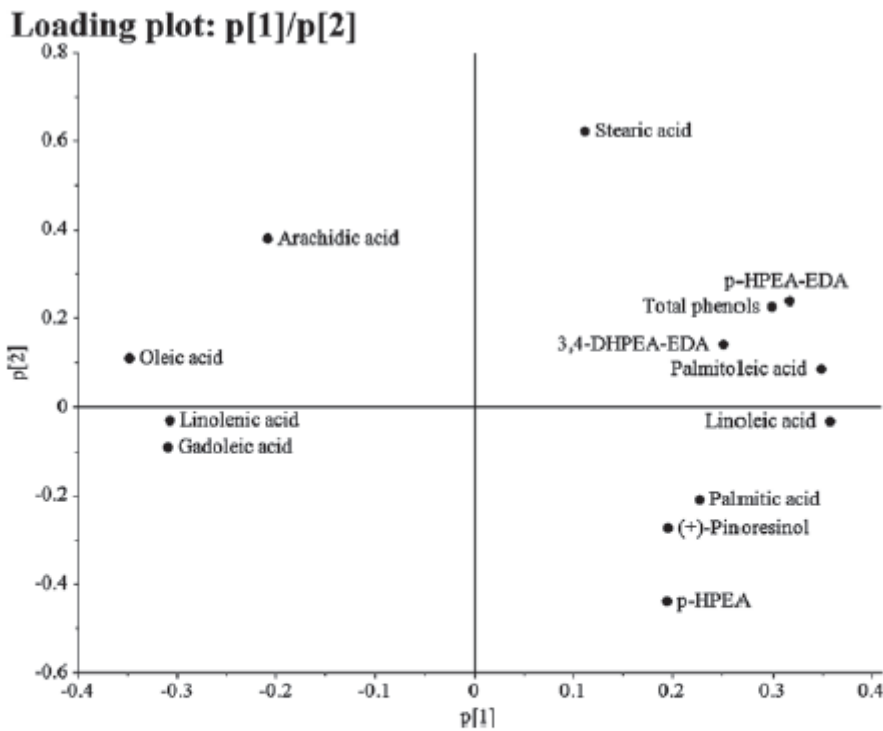
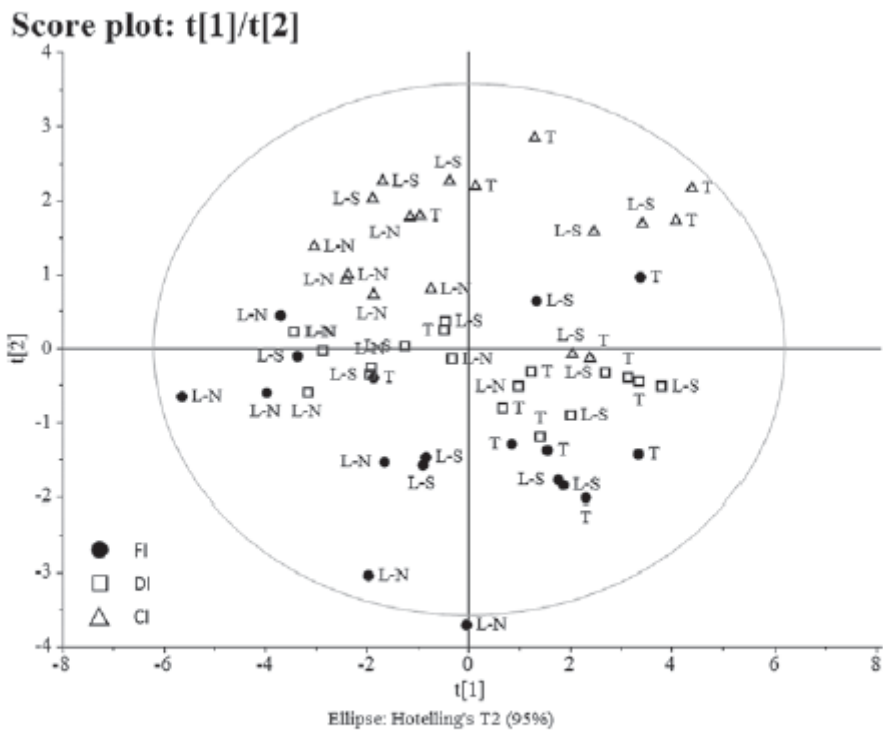
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**Figure 5.** Score plot and loading plot of the first two principal components of the PCA model built with all the virgin olive oils obtained in the 2 years using fatty acids and phenolic composition as variables. Different symbols in the score plot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