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# Olive and olive oil quality after intensive monocone olive growing (Olea europaea L., cv. Kalamata) in different irrigation regimes

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## Abstract

This work reports the results of a study carried out in an intensive monocone orchard of *Olea europaea* L. cv. Kalamata, a dual purpose (olive oil and table olives) variety, to investigate the influence of different irrigation regimes on productivity and quality of olives and olive oil. Irrigation regimes did not affect the sugar composition of the fruit, while the content of the phenolic compounds varied. In the water stress condition, olive fruit showed an higher cuticular thickness to prevent the loss of water and nutrients. Olive oil composition did not change with irrigation, except for the total phenols, which decreased. A restitution of 66% of crop evapotranspiration (ETc) was sufficient to achieve good yields, while higher water volumes (100% of ETc) gave little additional yield increases.  $\oslash$  2002 Elsevier Science Ltd. All rights reserved.

Keywords: Irrigation; Olea europaea L.; Olive oil; Quality; Table olives; Vegetative-productive growth

# 1. Introduction

In recent years, the consumption of olive oil and table olives has steadily increased, even in countries that do not have such a tradition. This trend is being fostered by the recognized nutritional value of the Mediterranean diet. To meet the increasing demand, olives are being produced in countries that do not have a tradition of olive growing, while models for intensive olive cultivation are being introduced in traditional areas to increase production and limit costs (Fontanazza, 1993). One model is based on the monocone growth form with a planting density of 400–500 trees per hectare depending on the vigor of the cultivar used in the olive orchard.

In areas with a Mediterranean climate, characterized by little or no rainfall during the most critical phenological phases for yield formation, intensive olive growing is barely feasible without irrigation. However, scant and contradictory data are available on the amount of seasonal water, necessary to obtain quality–quantitatively good productions from different olive cultivars. Such

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differences are probably due to varying degrees of cultivar adaptability to the pedoclimatic conditions and agronomic practices adopted in the field trials (Dettori, Filigheddu, & Scirra, 1989; Patumi, d'Andria, Fontanazza, Morelli, Giorio, & Sorrentino, 1999). Conflicting data have also been reported on the effect of irrigation on the olive fruit ripening and fatty acid composition (Gatto, 1989; Milella & Dettori, 1987; Wodner & Lavee, 1991), as well as on the quantity of polyphenols in the oil (Ismail, Stavroulakis, & Metzidakis, 1999; Motilva, Romero, Alegre, & Girona, 1999; Stefanoudaki-Katzouraki & Koustsaftakis, 1992). Such compounds are of great interest because they influence the quality and the palatabilty of the olive oils and prolong their shelf-life by slowing the formation of hydroperoxides of polyunsaturated fatty acids (Salas, Pastor, Castro, & Vega, 1997).

For table olives, irrigation is particularly important since it assures better vegetative and reproductive growth and guarantees a continuous, homogeneous increase of fruit size, together with a positive effect on fruit uniformity. Such properties are generally used to compare and distinguish different varieties, different degrees of ripeness and the effects of storage and processing.

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The present work reports production data and characteristics of olive fruits and oils obtained from trees irrigated with different water volumes.

## 2. Materials and methods

# 2.1. Plant material

Olive trees of Olea europaea L. cv. 'Kalamata', an oil and pickling cultivar, were tested in 2 years (1997 and 1998) of study. The field experiment was carried out at the CNR Irrigation Institute experimental farm near Benevento ( $14^{\circ}43'E$ ,  $41^{\circ}06'N$ ; elevation 250 m), a typical olive-growing area of southern Italy. The orchard was planted in 1992 with 1-year-old plants, grafted in a nursery on DA12 I $\degree$  clonal rootstock, patent IRO-CNR no. 1164/NV. Trees were clean-cultivated and trained using the monocone system (Fontanazza, 1993, 1994) at a planting density of  $6\times3$  m. The sandy-loam soil of the experimental site was characterized by a volumetric water content of  $35.6\%$  at field capacity (-0.03 MPa) and 21.2% at wilting point (1.5 MPa).

Olive trees were tested in a factorial combination with four irrigation levels: a rain-fed control  $(T_0)$  and three treatments  $(T_1, T_2, T_3)$  that received seasonal water amount equivalent to 33, 66 and 100% of crop evapotranspiration (ETc).

The experimental design was a complete randomized block, replicated four times. Each plot consisted of seven trees surrounded by ''strip'' trees to avoid interferences among treatments. ETc was estimated according to class 'A' pan evaporation (Doorenbos & Pruitt, 1977) from a meteorological station adjacent to the experimental field. Pan evaporation data were adjusted with a pan coefficient ( $k_p=0.8$ ), a crop coefficient ( $k_c=0.6$ ) and a tree ground-cover coefficient ( $k_r$  = 0.33 and 0.50 for 1997 and 1998, respectively), estimated according to Vermeiren and Jobling (1980). Irrigation water was delivered daily, 4 l/h/tree using a system with four drip nozzles (two per side), set in a line along the rows at a distance of 0.50 and 1.00 m from the trunk. The fruits were harvested in the first decade of November, when they were suitable for table consumption; fruit mean weight and fruit number per tree were determined, while fruit ripeness was evaluated according to Piedra (1987). Oil content and oil quality were also analysed on a representative sample of olives (3 kg per plot). In the second year, chemical composition, mechanical and ultrastructural properties of olive fruits were studied.

# 2.2. Fruit analyses

## 2.2.1. Mechanical test

For an objective assessment of fruit texture, a penetration test (Brighigna, Marsilio, & De Angelis, 1978–1980) was carried out, using a simple, inexpensive instrument (Effegi, Alfonsine, Ravenna, Italy). The penetration test was performed on 10 olives using a flat 2 mm  $\varnothing$  plunger which penetrated pulp at its equator, at a velocity of 120 mm/min. Four readings were taken on each verticallypositioned fruit that was cut around the equator. The mean of these values was determined and provided a measure of pulp firmness, interpreted as the maximal force  $(N)$  needed to push the probe 5 mm into the pulp.

## 2.2.2. Scanning electron microscopy

Tissue blocks (approx.  $3 \times 3 \times 1.5$  mm) of fresh olives were fractured revealing longitudinal views of the epicarp and mesocarp. The fracture face was cut away from the rest of the slice and fixed with 3% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) for 4 h at room temperature. The samples were washed with the same buffer, dehydrated in alcohol series (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% ethanol), and critical pointdried. The dry tissues were then mounted on aluminium stubs and coated with gold (25 nm thick) in a Sputter Coater. Representative specimens were examined at 20 kV with a Philips SEM XL 20, equipped with Image Analysis to obtain measurements of different olive tissues and then photographed with a Pentax camera. The cuticular minimum thickness was measured from the cuticle surface to the top of epidermal cell; the cuticular maximum thickness was measured from the cuticle surface to the base of the epidermal cell.

#### 2.2.3. Organic acid and sugar determinations

The analyses were performed according to the method described in a previous work (Patumi et al., 1999).

## 2.2.4. Determination of total phenols

The amounts of total phenolics in the fruit extracts were determined colorimetrically using the Folin-Ciocalteu procedure (Singleton & Rossi, 1965). The absorbance was measured at 725 nm (Perkin-Elmer  $\lambda$ 2 UV/ VIS spectrophotometer, Norwalk, CT) and data expressed as ppm of gallic acid.

# 2.2.5. Determination of phenolilc compounds by GC and GC-MS analyses

Fruits, destoned and immediately frozen in liquid nitrogen, were triturated in a blender. Approximately 5 g of the powder obtained were homogenized four times in 30 ml of 80%  $(v/v)$  ethanol, containing 0.5% sodium metabisulfite, and centrifuged at 5000g at  $2-3$  °C for 20 min. An ethanolic solution of resorcinol (0.5 g/l) was added as internal standard. The combined supernatants were concentrated under reduced pressure and washed with hexane. The remaining aqueous solution, partitioned four times with ethyl acetate in a water to phase ratio of 1:1, was filtered on sodium sulphate (anhydrous) and evaporated to dryness at  $30^{\circ}$ C under

vacuum. The dry residue was converted into trimethylsilyl derivatives with a silylation mixture made up of pyridine, hexamethyldisilazane and trimethylchlorosilane (2:1:1) for 1 h at room temperature. The silanized extracts were dried, dissolved in isoctane and further analyzed by GC and GC-MS. A Carlo Erba GC-5160, equipped with a FID and a HP 1 capillary column (Hewlett-Packard, Palo Alto, CA) of 30 m  $\times 0.32$  mm (i.d.), 0.10 µm film thickness, was used for GC analyses. The column temperature was programmed from 70 to 90 °C at 20 °C/min, from 90 °C to 300 °C at 4 °C/min and held at 300 °C for 40 min. Hydrogen was used as carrier gas at 35 kPa. The sample  $(0.3 \text{ µl})$  was injected by the "on column" mode. A Hewlett-Packard GC-5890 interfaced to a MSD-5970 was used for GC-MS analysis. The chromatographic conditions were the same as described above. EI-MS were obtained at 70 eV, with helium as carrier gas. Compounds extracted by ethyl acetate were identified by comparing both their retention times and mass spectra with those of authentic compounds or reference standards.

# 2.3. Oil analyses

## 2.3.1. Oil content and oil extraction

Oil content was determined by extracting dry material with  $40-60$  °C petroleum ether using a Soxhlet apparatus (Donaire, Sanchez-Raya, Lopez-George, & Recalde, 1977). Olive oil used for the qualitative analysis was obtained using a bench hammer mill: the crushed fruits were mixed for 30 min at 25  $\degree$ C, and then the oil was separated by centrifugation.

# 2.3.2. Fatty acid analysis

The analyses were performed according to the method described in a previous work (Patumi et al., 1999).

#### 2.3.3. Determination of total phenols

The amount of total phenolics in oil was determined according to the Folin-Ciocalteu procedure, as described for fruit extracts.

# 2.3.4. Organoleptic evaluation of oils

Organoleptic evaluation of oil was carried out by panel test, using the I.O.O.C. (International Olive Oil Council, 1996) methodology, in which both defect and merit intensities of oils are reported on a conventional scale between 0 and 100 mm. The initial point of the linear scale (0 mm) represents the absence of the organoleptic characteristics analyzed, while the final point (100 mm) represents the highest value in the evaluation of a given set of oil standards, according to the I.O.O.C.



Fig. 1. Minimum and maximum air temperature (10-day mean) and rainfall (10-day sum) in (a) 1997 and (b) 1998 compared with 1982– 1998 mean.



Table 1 Seasonal irrigation volumes and useful rainfall (rainfall  $>10$  mm in 24 h)<sup>a</sup>

<sup>a</sup> T<sub>0</sub>=rainfed control; T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>=irrigated treatments with 33, 66 and 100% of ETc, respectively b Rainfall throughout the irrigation season of each experimental year is included.





<sup>a</sup> T<sub>0</sub> = rainfed control; T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> = irrigated treatments with 33, 66 and 100% of ETc, respectively. b Last significant difference (P=0.05) test.

#### 2.4. Data analysis

Data were subjected to the analysis of variance and mean separation was obtained using the least significant difference (LSD) test.

## 3. Results and discussion

The 2-year experimental period was characterized by different rainfalls. In 1997, there was a notable quantity of rain in July and again in October, before harvest, while in 1998 rainfall was below the 16-year mean in the period May–November (Fig. 1). These climatic conditions influenced the applied seasonal irrigation volume (Table 1).

Plant productivity was positively affected by irrigation (Table 2). In the first experimental year an irrigation volume of  $66\%$  of ETc  $(T_2)$  showed a significantly higher yield than the rainfed control  $(T_0)$ ; however differences were not evident between treatments  $T_2$  and  $T_3$ . In the dry climate of 1998,  $T_1$  achieved a higher yield than  $T_0$ , as well as  $T_3$  versus  $T_2$ , although differences between the last two treatments were not significant. The increased yield was due, both to the increase in mean fruit weight, and to fruit number per tree. In particular, in 1998, characterized by lack of rainfall during the summer, fruit mean weight of treatment  $T_0$  was 42% lower than T<sub>3</sub>, while, in 1997, differences were less evident due to the abundant precipitation during the final period of fruit growth  $(T_0$  was about 11% lower than  $T_3$ ).

Fruit yield indicated that when the rainfall was near the polyannual trend, an irrigation treatment corresponding to 66% of ETc was sufficient for a production similar to that obtained from trees irrigated with a

water volume corresponding to 100% of ETc. Such results are in agreement with other experiments conducted on the same (Michelakis, Vouyoukalou, & Clapaki, 1995) and different (Chartzoulakis, Michelakis, & Tzompanakis, 1992; Milella & Dettori, 1987) cultivars in other environments. Compared with the control  $(T_0)$ , 33% of ETc improved yield only in the driest year. Therefore, according to Milella and Dettori (1986), the climatic trends of a particular year can influence the productive response of an olive grove. The decreased yield recorded by  $T_0$  in 1998, compared with that of 1997, could also be due to an alternate bearing phenomenon accentuated by the dry climate conditions (Psyllakis, 1976). In fact, this behaviour was not recorded in the irrigated treatments. The irrigation regime did not cause any variation in oil accumulation in the fruit; therefore, oil production reflected the fruit yield pattern (Table 2). Sugars are the main water-soluble components in olive pulp (Donaire et al., 1977). They play an important role by providing energy for metabolic changes; they constitute cell-wall components responsible for the fruit texture (Jimenez, Guillen, Sanchez, Fernandez-Bolanos, & Heredia, 1995) and act as a carbon source for micro-organisms involved in the fermentation during table olive processing. Sugar content (Table 3), expressed in% on a dry weight basis, ranged from 7.08  $(T_0)$  to 7.88%  $(T_3)$ . Differences between irrigated treatments  $(T_1, T_2 \text{ and } T_3)$  were not significant. Glucose, galactose and fructose were the predominant components, while sucrose was detected in very low concentrations. The contents ranged from 0.55 to 0.79%, 0.95 to 1.14% and 4.05 to 4.33% for fructose, galactose and glucose, respectively. Generally glucose accounted for 56%, fructose for 9% and galactose for 14% of the total sugars. Mannitol was also found, with a content ranging between 1.28 and 1.48% (about 18%



Irrigation level	Sugar content $(\%$ DW)						Acids content $(\%$ DW)		
	Fructose	Galactose	Glucose	Sucrose	Mannitol	Total	Malic	Citric	Total
$T_0$	0.57	0.95	4.05	0.21	1.30	7.08	1.21	1.18	2.39
$T_1$	0.79	1.06	4.20	0.15	1.28	7.48	1.18	1.33	2.51
T <sub>2</sub>	0.55	1.00	4.33	0.22	l.48	7.58	1.27	1.36	2.63
$T_3$	0.72	1.14	4.31	0.24	l.47	7.88	.30	1.27	2.57
LSD <sub>0.05</sub> <sup>a</sup>	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 3 Sugar and acid contents of fruit as a function of irrigation level

<sup>a</sup> Last significant difference ( $P=0.05$ ) test.

of the total sugars). Organic acids, such as malic and citric, were also found in the fruit extracts (Table 3). Such acids are important contributors to the colour of table olives and influence the processing treatments (Marsilio, Vlahov, & Brighigna, 1978–1980) by affecting buffering capacity of olive tissues. Their contents were around 1.2–1.3% and significant differences between treatments were not evident.

Among hydrosoluble components, polyphenols also play an important role in olive-fruits since they have a wide range of biochemical and pharmaceutical effects, including anticarcinogenic, antiatherogenic, antimicrobial and antioxidant activities (Kohyama, Nagata, Fujimoto, & Sekiya, 1997; Visioli & Galli, 1998). Factors, such as olive cultivars and ripeness stage, cultivation areas, seasonal climatic variations, and agronomic practices, influence the phenol content in olive-fruit (Amiot, Fleuriet, & Macheix, 1986). In table olive processing, a balanced phenolic profile is important to control microbiological spoilage, by inhibiting cell walldegrading enzymes, and to guarantee a distinctive flavour and taste of the product (Marsilio & Lanza, 1998; Marsilio, Lanza, & Pozzi, 1996).

Phenolic compounds, both in the fruit and in the oil, were negatively affected by irrigation up to a volume of 66% of ETc (Table 4). This behaviour cannot be attributed to a different degree of fruit ripeness (Amiot, Fleuriet, & Macheix, 1986) since fruits showed a uniform level of ripening.

The major phenolic compounds found in olive fruits are shown in Fig. 2. Compared to the rainfed control  $(T_0)$ , such compounds decreased in  $T_3$  with the exception of hydroxytyrosol, which slightly increased.

Fruit turgidity, in terms of firmness, measured by the penetration test, was higher in the irrigated treatments than rainfed control. Values ranged between 1.15 and 1.96 N for treatments  $T_0$  and  $T_3$ , respectively (Fig. 3).

Microstructural observations of olive tissues indicated that the irrigation treatments affected the cuticular layer. The fruit cuticle (skin), a continuous layer connected to the epidermal cells, consisting of pectopolysaccharides (pectin, cellulose and hemicellulose), cutin and wax layers (Fig. 4) (Jeffree, Baker, & Holloway, Table 4

Total polyphenols in the fruit and in the oil as a function of irrigation levela

Irrigation level	Fruit phenols (ppm)	Oil phenols (ppm)	
1997			
$T_0$	22744	253	
$T_1$	21778	190	
T <sub>2</sub>	18583	160	
T <sub>3</sub>	18026	166	
$LSD0.05$ b	908	18	
1998			
$T_0$	19072	227	
$T_1$	19354	213	
T <sub>2</sub>	18070	160	
T <sub>3</sub>	17942	150	
$LSD0.05$ <sup>b</sup>	780	14	

<sup>a</sup> T<sub>0</sub>=rainfed control; T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>=irrigated treatments with 33, 66 and 100% of ETc, respectively.

<sup>b</sup> Least significant difference  $(P=0.5)$  test.

Table 5 Cuticular thickness of olive fruits as a function of irrigation level<sup>a</sup>



<sup>a</sup> T<sub>0</sub>=rainfed control; T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>=irrigated treatments with 33, 66 and 100% of ETc, respectively.

<sup>b</sup> Each value is the mean of 10 determinations  $\pm$  standard deviation.

1976; Mafra et al., 2001), varied greatly, depending on environmental factors, such as humidity, light and temperature. The cuticle has been referred to as the barrier between the intracellular and extracellular environment which prevents the diffusion of water and nutrients from the fruit (Martin & Juniper, 1970). Table 5 shows a reduced cuticular thickness going from  $T_0-T_1$  to  $T_2-T_3$ , probably due to the overcoming of a critical irrigation level (treatment  $T_2$ ) at which the plant develops specific defence systems.





<sup>a</sup> Saturated acids: C:16 palmitic, C:17 heptadecanoic, C:18 stearic, C:20 eicosanoic; unsaturarted acids: C":16 palmitoleic, C":17 heptadecenoic, C':18 oleic, C":18 linoleic, C"':18 linolenic eicosanoic, C':20 eicosenoic. uns/sat = unsaturated saturated acids ratio; mono/poly = monounsaturated polyunsaturated acids ratio.

 $\frac{1}{2}$  T<sub>0</sub> = rainfed control; T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> = irrigated treatments with 33, 66 and 100% of ETc, respectively. <sup>c</sup> Last Significant Difference (*P* = 0.05) test.



Fig. 2. Phenolic compounds of olive pulp from  $T_0$  (rainfed control) and  $T_3$  (100% of ETc) treatments.



Fig. 3. Results of the penetration test. Each value is the mean of 10 determinations  $\pm$  standard deviation.

In  $T_0$  (Fig. 5), the cuticle was thick and appeared as an amorphous layer. The same pattern was found in  $T_1$ although some cellulose microfibrils were seen embedded in the cutin matrix. The cellulose reticulum was



Fig. 4. Scheme of olive cuticle.

more evident in  $T_2$  and  $T_3$ , where the cutin content is lower with a consequently thinner cuticle. These results could be explained as an adaptation of the plant to the climate conditions. In the absence or scarcity of water the plant produces fruits with a higher cuticular thickness and repellent layers (wax and cutin) to prevent the loss of water and nutrients, whereas, in humid conditions, the fruit cuticle becomes thin.

The irrigation regime barely affected the oil fatty acid ratios or composition (Table 6). The unsaturated/saturated acid ratio influences the organoleptic characteristics of the oil because an oil with a high content of saturated fatty acids is more viscous and persistent in the mucous of the oral cavity. This gives rise to the defect defined as a ''fatty sensation'' (Solinas, 1990). Also, the monounsaturated/polyunsaturated acid ratio, around 10, did not appear to be influenced by irrigation, and therefore the intrinsic oxidative stability of the oil remained unchanged (Lerker & Capella, 1997).

Acidity and peroxide values of oils (Table 7) were low in all treatments, being below 0.45%, as oleic acid and 4.8 meq  $O_2$  kg<sup>-1</sup>, respectively. After 10 months of storage, the acidity did not vary, while the peroxide value increased, but values were below the legal limit (20 meq  $O_2$  kg<sup>-1</sup>) for extra-virgin olive oil quality.

Sensory characteristics, tested by I.O.O.C. methodology only in  $T_0$  and  $T_3$  treatments (Table 8), showed no defects in any of the oils and they are classified as extravirgin olive oils. Oils from the irrigated regime were less bitter  $(-40\%)$  and less pungent  $(-25\%)$  than those of



Fig. 5. Scanning electron micrographs of olive cuticle: (a)  $T_0$  = rainfed control; (b)  $T_1$ =33% of ETc; (c)  $T_2$ =66% of ETc; (d)  $T_3$ =100% of ETc.

Table 7 Acidity and peroxide number of oil as influenced by irrigation

Irrigation level	Acidity $(\%$ oleic acid)	Acidity <sup>a</sup> $\frac{6}{6}$ oleic acid)	Peroxide number (meq $O_2/kg$ )	Peroxide number <sup>a</sup> (meq $O_2/kg$ )	
$T_0$	0.37	0.45	4.5	7.4	
$T_1$	0.35	0.41	4.7	8.1	
T <sub>2</sub>	0.35	0.42	4.6	9.7	
T <sub>3</sub>	0.41	0.45	4.8	8.5	
LSD <sub>0.05</sub>	ns	ns	ns	ns	

<sup>a</sup> Data analysed 10 months after extraction.

<sup>b</sup> Last significant difference ( $P=0.05$ ) test.





the control, while the fruity sensation was the same. Therefore, irrigation enhances the organoleptic characteristics of the oil of this cultivar, particularly appreciated for the absence of bitter and pungent tastes.

In conclusion, results of this study indicated that yield and fresh fruit quality of the cultivar Kalamata are positively affected by irrigation. A restitution of 66% of ETc was necessary to achieve good yield, while higher volumes (100% of ETc) do not further enhance yields. Irrigation regimes did not affect acidic composition or

shelf-life of the oil. The decrease of polyphenol content in the fruit was not detrimental to oil organoleptic characteristics. Moreover, the better textural properties registered in fruits from irrigated plants entail a good maintenance of fruit characteristics, both for table olive production and for olive oil extraction.

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