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Influence of Fruit Load and Water Deficit on Olive Fruit Phenolic Profiling and Yield

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Abstract: High-density olive groves, despite their interesting production potential, have several limitations, also due to the high fruit load and the irrigation requirements. The study aimed to evaluate the effects of fruit load and deficit irrigation on oil yield and fruit quality, and on olive chemical composition in a high-density olive grove (cv Sikitita). Our main hypothesis was that the primary metabolism, as influenced by crop load and stress, could modify the accumulation of different phenolic classes. Different fruit loads were generated through flower thinning (66%, 50%, 33%, 0%) and two deficit irrigation treatments (-60%, -75%) were compared to the well-watered control (920 m3/ha). Thinning treatments had a limited effect on oil yield, on the other hand, deficit irrigation caused considerably less oil accumulation in the fruit on all sampling dates. Thinning 66% and deficit irrigation 75% were considered with the control for untargeted metabolomic analysis, considering three sampling dates. 233 different phenolic compounds were annotated. Multivariate HCA results indicated the impact of harvest time on the phenolic profile of olive fruits, yielding two separate clusters grouping t1 and t2 together and apart from t3. Regarding agronomic techniques, they played a differential role in the phenolic profile (supervised OPLS – DA). Fruit load mostly affected flavonoid glycosides. In contrast, the phenolic response to deficit irrigation is more heterogeneous, with phenolic acids (35%), flavonoids (25%), LMW, and other phenols (25%).

Keywords: crop load; deficit irrigation; metabolomics; secondary metabolites; phenolic compounds

1. Introduction

Extra virgin olive oil (EVOO) worldwide consumption and production are increasing due to its now well-known health properties. Italy is one of the most important players in the olive oil sector [1–4] but, in this country, consumption is greater than its production, and consequently, some quantities of olive oil must be imported [5]. The Italian olive oil sector faces structural [3,6], olive grove management, and climate change [7] issues that limit its sustainable economic development, leading to the loss of competitiveness and market share of its main competitors. Indeed, Italian olive growers have production costs 1.30 times higher than the international weighted average cost of obtaining one kilogram of olive oil [8]. On the other hand, Italy has the richest olive biodiversity in the world [6] with about 600 varieties [9], and this allows for a unique number of cultivar-territory combinations and consequently numerous products, including many premium products. In Italy, oil quality is probably the only viable strategy for competition in the market [10]. However, it is necessary to optimize production costs too. In this perspective, the super-high-density olive orchards are the most productive and can produce oil at competitive prices to cope with the increasing global demand. However, the super high-density olive groves have some limitations [11], such as low oil yields in olive and high fruit water content, that could lead to the formation of emulsions during the oil extraction process, resulting in decreased oil extractability [12]. Moreover, oil yield, intended as the oil accumulation in the fruit, is affected by several factors, including environmental conditions [13], crop load [14,15], crop management and irrigation [16,17].

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The quantity of fruits on the tree is a crucial factor that significantly influences the oil yield and primary metabolism. Several studies have established that elevated crop loads frequently result in reduced fruit size, decreased mesocarp: endocarp tissue ratios, and lower oil content per individual fruit [18]. To the best of our knowledge, few studies investigate the effects of fruit load on fruit quality traits and chemical composition.

In terms of quality, phenolic compounds are abundant secondary metabolites of olives, renowned for their functional role and ability to scavenge free radicals [19] and their role in plant response to stress conditions [20]. This latter response is complex and encompasses a network of metabolic pathways leading to various metabolite adjustments [21]. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) 2011 [22], found that olive polyphenols (olive fruit, olive mill waste waters or olive oil, Olea europaea L. extract and leaf) standardized to hydroxytyrosol content and its derivatives (5 mg in 20 g of olive oil) have a beneficial effect on human health (e.g., contribute to the protection of blood lipids from oxidative stress). However, the results by Jerman Klen & Mozetič Vodopivec, 2012 [23], indicated that the qualitative profile of olive phenols is significantly different from that of oil, suggesting that phenols are not only transferred but also transformed during oil processing. Indeed, the main fruit secoiridoids, such as oleuropein, demethyloleuropein, and ligstroside, and many fruits' hydroxytyrosol glucoside were degraded during crushing/malaxation operation, forming several secoiridoid aglycone derivatives and hydroxytyrosol. However, more phenols in the fruit would automatically lead to more phenols in the oil [24].

The interaction between the genetic background (the cultivar) and environmental conditions can provide distinct phytochemical profiles in the fruits. In a study by Ortega-García & Peragón, 2009 [25], the Frantoio cultivar was found to be the variety with the highest phenolic content, compared to Picual, Verdial, and Arbequina cvs. Nevertheless, agronomic practices, water availability, and irrigation management have strongly affected olives' phenolic composition [26]. Indeed, water deficit further limits carbon assimilation, and primary metabolism is widely recognized to impact water productivity of drupe metabolism, consequently affecting key chemical traits of the fruit [27]. Water deficit can alter the levels of compounds associated with oxidative imbalance, such as the polyphenols oleuropein, verbascoside, luteolin 7-O-glucoside, and apigenin 7-O-glucoside [21].

In literature, the quality of oil in response to different irrigation strategies has been widely investigated [16,17,28–31], including olive chemical composition [21,26,27,31–34]. Nevertheless, there is a gap in knowledge regarding the accumulation of the different phenolic classes within the fruits in response to events that may alter the primary metabolism, such as fruit load and deficit irrigation.

Our main hypothesis was that the effect of fruit load and water deficit on primary metabolism may influence phenol accumulation. Consequently, several biosynthetic pathways in olives, particularly that of shikimate, phenylpropanoids, and mevalonate, may be more affected.

Therefore, this work aimed to study the influence of fruit load and deficit irrigation on the main sinks of the olive tree, vegetative growth, and yield, and on the phenolic profile of olives over-ripening. Specifically, this was achieved by (a) evaluating the effects on the olive growth dynamics, the ripening, and the oil accumulation; (b) investigating, through an untargeted metabolomics approach, the fruit phenolic profile in response to fruit load and deficit irrigation; and (c) identifying by multivariate statistics the phenolic compounds mostly involved in response to both agronomical practices.

2. Materials and Methods

2.1. Experimental Orchard and Experimental Design

The study was conducted in 2021 in a high-density 4-year-old olive grove of Sikitita cultivar (University of Córdoba and the Institute of Agricultural and Fishery Research and Training (IFAPA), 1996) with trees planted at 1.5 m on the row and 4 m between rows. The olive grove was located near Florence (43.763029, 11.416663, Central Italy), at an elevation of 180 m above sea level. The farm weather shed recorded an average temperature of 18.2 °C and rainfall of 377.9 mm from March 1st, 2021, to November 1st, 2021. Referring to the irrigation period, which began on May 1st and ended on September 31st, 244 mm of rainfall was recorded.

 doi:10.20944/preprints202404.1440.v1

Different fruit load levels were set by applying different levels of flower thinning (66%-one out of 3-, 50%-1 out of 2-, 33%- 2 out of 3- of inflorescences removed by hand at BBCH 55 from the whole tree canopy) in order to reduce, as much as possible, the effect of flower and fruit set formation on the primary metabolism of the tree.

Figure 1 were selected to carry out thinning: ten trees were randomly assigned to the following flower thinning treatments: 66%, 50%, 33%, and 0% (unthinned, control). Twenty more trees, on the same rows (Figure 1), were selected for irrigation reduction. Ten control trees, the same ones selected for the thinning treatment, were irrigated according to the farm standard irrigation (920 m3/ha). Ten trees per treatment were randomly assigned to the two deficit irrigation treatments: 60% reduction (383 m3/ha) with a 2-L/h dripper per plant and 75% reduction (230 m3/ha) with a 1.2-L/h dripper per plant. Irrigation was calculated to restore 100% of the required water demand, obtained by the equations previously described by Sastre et al., 2022 [31]. Therefore, the FAO method [35] was used to estimate crop evapotranspiration under standard conditions (ETc). Reference crop evapotranspiration (ET0) was determined with the Penman-Monteith method and was 589 mm between May 1st and August 31st. The crop coefficient (Kc) of 0.70 was selected from the FAO manual [35] as typical and recommended for olive trees. Considering that the alley was covered by an active ground cover, the Kc value was added by 0.25, as usually recommended by FAO for other tree crops such as apple, pear, and cherry. The coefficient (Kr) which links the degree of orchard vegetation cover to evapotranspiration was found to be 0.50, with olive trees covering 25% of the orchard area.

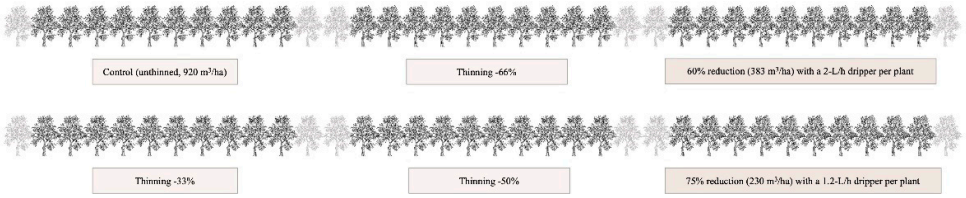

Figure 1. Experimental design.

2.2. Field Measurements and Fruit Harvest

Each month, from full bloom (May 21st, 2021) to the initial phase of ripening (October 7th, 2021), vegetative growth was monitored: the shoot length (cm), the shoot nodes (number/shoot), the number of inflorescences (number/shoot), the fruits set (number/shoot), and the number of fruits (number/shoot) were measured on a representative two-year-old shoot per tree. In addition, the current year shoot length (cm), and its number of nodes (number/apical shoot) were also recorded. In full bloom, before applying the different treatments, the trees showed similar vegetative and productive situations (Table S1). Every month, from the fruit set (June 23rd, 2021) to the harvest time (November 15th, 2021), eight samplings were collected, and ten fruits were randomly sampled from five plants per treatment. Fresh weight, dry weight, pulp: stone ratio, maturation index, and oil accumulation (g) were monitored on thirty fruits per treatment. Part of the collected samples (twenty fruits) were frozen for metabolomics analysis. For deficit irrigation treatments, stem water potential (Ψstem) was measured over the same days, from late June to early October, using a pressure chamber (Soilmoisture Corp, Santa Barbara, CA, USA). Ψstem was measured on each olive tree on one mature leaf, which had been wrapped in aluminum foil two hours before measurements [36]. The olives were harvested in two stages: at an intermediate ripening stage (October 25th, 2021) and at a more advanced ripening stage (November 15th, 2021).

2.3. Maturation Index and Fruit Yield

The maturation index was determined according to the procedure proposed by the International Olive Council by evaluating the color of the skin and the pulp of the olives [37]. Oil extraction from the olive samples was done using the Milestone ETHOS X microwave. For each olive sample, the pulp was separated from the stone before drying in an oven at 50 degrees for at least 12 hours and crushing. Then, 1 g of sample was placed in the vessel with 25 mL of Petroleum ether. The combined

 doi:10.20944/preprints202404.1440.v1

hydrolysis and extraction process took place at 125 °C and required, approximately, 50 minutes. The temperature was monitored and controlled in each vessel using a unique combination of fiber optic and infrared temperature sensors. The ETHOS X built-in magnetic stirrer assured an even heating and temperature uniformity in all vessels, as well as intensive mixing between the aqueous and the solvent phases. The heating phase was followed by a 15-20 min cooling phase. The cooled samples were dropped into 50-mL Falcon tubes and centrifuged for 6 minutes at 5,000 g. The supernatant was then transferred into weighed disposable cups. The measured aliquot of the solvent with the extracted oil inside was evaporated under a fume hood. Finally, the individual cups with the extracted oil inside were weighed and the oil yield was expressed on the fresh weight of the whole drupe.

2.4. Sample Preparation and Extraction for Metabolomic Analysis

For the metabolomics investigation, olive samples from the control, 66% thinning, and 75% irrigation reduction treatments, collected in early September (September 2nd, 2021), late September (September 23rd, 2021), and mid-November (November 15th, 2021), with n=5 biological replications, were selected. Samples extracts were obtained by mixing 1 g of fresh olive pulp with 10 mL of 80% aqueous methanol solution (v/v) acidified with 0.1% formic acid (v/v). The mixture was homogenized by Ultra-Turrax high-speed rotor for 1 minute and then centrifuged at 6000 g for 10 minutes at 4° C (5190-R, Eppendorf). After centrifugation, 1-mL aliquots were collected from the resulting supernatants, further filtered using a cellulose syringe filter of 0.22 µm pore size and transferred to vials for metabolomics analysis.

2.5. Phenolic Profiling Using Untargeted Metabolomics

Phenolic compounds of olive extracts were profiled through ultra-high-performance liquid chromatography coupled to a high-resolution quadrupole-time-of-flight hybrid mass spectrometry approach (UHPLC/QTOF-HRMS), as previously described [38]. The chromatographic system (UHPLC 1290 instrument by Agilent® Technologies, CA, USA) was equipped with a reverse-phase Agilent® Poroshell 120 PFP column (100 mm × 2.1 i.d., 1.9 µm pore size), and the mobile phase consisted in a binary mixture of ultrapure water (solvent A) and acetonitrile (solvent B). Both solvents were acidified with 0.1% formic acid (v/v) and the chromatographic separation was achieved by applying a continuous gradient elution (6%B to 94%B) for 32 min, followed by an equilibration period of 3 min to return to the initial elution conditions before the subsequent injection. The flow rate was adjusted at 200 µL min^{-1} and the injection volume was 6 µL.

The QTOF-MS analyzer (iFunnel G6550, Agilent®) was equipped with an electrospray ionization source. The mass spectrometer operated in positive polarity (ESI+) and SCAN modes, with an acquisition range of 100 – 1200 m/z (extended dynamic range) with a resolution of 30,000 FWHM. Nitrogen was employed as sheath gas (12 L min^{-1}, 315 °C) as well as drying gas (14 L min^{-1}, 250 °C). Nebulizer pressure was adjusted at 45 psi, and nozzle and capillary voltage were set at 350 V and 4,000 V, respectively. After the acquisition, raw features deconvolution was carried out from the total ion current and further processed by the Agilent® MassHunter Profinder software (v.10.0). The annotation was carried out by the "find-by-formula" algorithm setting the following parameters: retention time and mass alignment, 0.1-min retention time tolerance, 5-ppm mass tolerance, and data reduction filter to remove the features that were not present in at least the 80% of replicates within each experimental group. Annotation was carried out by considering the isotope pattern of acquired features, involving their monoisotopic mass, isotopic spacing, and isotopic ratio. The database Phenol-Explorer 3.6 [39] was used to achieve the annotation of phenolic compounds, which was obtained following the level 2 (putatively annotated compounds) of the COSMOS initiative (Coordination Of Standards in MetabOlomicS) [40].

Once annotated, phenolic compounds were classified and grouped into classes and subclasses, and then semi-quantification was done using a representative analytical standard for each class (cyanidin for anthocyanins, (+)-catechin for flavanols, luteolin for flavones and other flavonoids, quercetin for flavonols, sesamin for lignans, tyrosol for the low-molecular-weight (LMW) and other

 doi:10.20944/preprints202404.1440.v1

phenolics, ferulic acid for phenolic acids, and trans-resveratrol for stilbenes). For this purpose, standards were used to build the corresponding calibration curves by preparing serial solutions analyzed through the same workflow used for the samples. All reference standards were analytical grade reference compounds (Extrasynthase®, Lyon, France). The results from the semi-quantification of samples were expressed as equivalents of the corresponding standards in mg per gram of fresh weight (mg g^{-1} FW).

2.6. Statistical analysis

The abundance of annotated compounds was transformed to $\log_2$, normalized at the 75th percentile, and baselined against the median values of all samples before applying multivariate statistical analyses using the software Mass Profiler Professional (v. 15.1, Agilent Technologies). Firstly, an unsupervised hierarchical cluster analysis (HCA) was performed to assess the influence of different factors on the phenolic profile of olive samples according to their profile-wide similarities. A fold-change-based heatmap was used to obtain the corresponding clustering dendrogram, applying Euclidean distance and Ward's linkage rule. Afterward, a fold change (FC) analysis was performed to assess the relative variation of each compound due to the different treatments with respect to control. In parallel, a supervised orthogonal projection to latent structures discriminant analysis (OPLS-DA), using the SIMCA software (v. 16.0, Umetrics®, Malmö, Sweden) was performed to discriminate the effect of every factor on the phenolic profile of olive samples. The quality of OPLS models was determined by goodness-of-fit and goodness-of-prediction parameters (R^2 and Q^2, respectively). Moreover, models were statistically validated by cross-validation analysis of variance (CV-ANOVA, $p < 0.05$) and overfitting was excluded according to the permutation test. Each OPLS model was combined with variable importance in projection (VIP) analysis to identify the most discriminant metabolites, setting a VIP score threshold of 1.4.

Concerning the data from the semi-quantification of phenolic compounds in olive samples, results were expressed as the mean ± standard deviation (SD), and statistically analyzed by one-way ANOVA followed by Duncan's post hoc test, assuming a significance threshold of $\alpha = 0.05$ (n = 5), using the software SPSS (v. 25.0, IBM®, NY, USA).

Water potential data were analyzed using Sigmaplot 8.0 (SystatSoftware Inc., San Jose, CA, USA). The data referring to the effects of fruit load and deficit irrigation on olives and oil accumulation are presented as mean ± standard error, statistically analyzed by one-way ANOVA followed by Tukey's post hoc test, assuming a significance threshold of $\alpha = 0.05$ (n = 5), using the software SPSS (v. 25.0, IBM®, NY, USA).

3. Results

3.1. Fruit Load and Deficit Irrigation Effect on Oil Accumulation, Water Potential, and Olive Growth Dynamics

Regardless of the treatment, a steady increase in oil yield over time was observed. Thinning treatments had an oil accumulation trend similar to the control, with no statistically significant differences until the last sampling time, with slightly higher yields attributed to 33% and 50% thinning (Table 1). Conversely, the deficit irrigation caused a meaningful decrease in oil accumulation within the fruit. A significant statistical difference in oil yield is observed between the deficit irrigation theses and the control, since September 2nd. (Table 1).

Concerning vegetative growth, at pre-harvest time (October 7th, 2021) number of fruits, length, and nodes of the current year shoot showed significant differences (Table S1). Specifically, current-year vegetative growth exhibited higher in theses with flower thinning than in the control. On the other hand, the water deficit resulted in a noticeable decrease in both vegetative growth and the number of fruits. Although the number of inflorescences at the beginning of the experiment (during full bloom on May 21st, 2021, as shown in Table S1) was higher in the deficit irrigation treatments -60% and -75% than in the other treatments, the water deficit resulted in significantly fewer attached fruits and a reduced fruit yield at harvest compared to the control. Furthermore, at the pre-harvest

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time, the thinning treatments led to a decrease in the number of fruits, and consequently an increase in the current year vegetative growth, compared to the control.

Table 1. Olive oil yield, provided as a percentage of weight with respect to olive fruits, over time according to the different treatments applied.

Treatments	Sampling Dates							
	06/23	**07/19**	**08/11**	**09/02**	**09/23**	**10/07**	**10/25**	**11/15**
Control	0.0% ± 0.00 a	1.4% ± 0.22 a	4.8% ± 0.61 a	11.8% ± 0.43 a	12.7% ± 0.80 a	14.7% ± 1.02 a	19.3% ± 0.40 a	22.3% ± 0.29 b
Thinning -33%	0.0% ± 0.00 a	1.3% ± 0.12 a	5.8% ± 0.33 a	12.6% ± 0.38 a	12.6% ± 0.59 a	15.7% ± 0.61 a	20.3% ± 0.87 a	23.6% ± 1.13 a
Thinning -50%	0.0% ± 0.00 a	1.2% ± 0.25 a	6.1% ± 0.47 a	11.9% ± 0.27 a	12.8% ± 0.72 a	14.8% ± 0.27 a	19.6% ± 0.84 a	23.8% ± 0.72 a
Thinning -66%	0.0% ± 0.00 a	1.0% ± 0.26 a	6.3% ± 0.29 a	12.5% ± 0.38 a	12.6% ± 0.71 a	15.0% ± 0.76 a	19.2% ± 1.47 a	21.6% ± 0.96 b
Irrigation -60%	0.0% ± 0.00 a	1.1% ± 0.38 a	6.6% ± 0.72 a	9.6% ± 1.31 b	12.7% ± 0.38 a	13.6% ± 0.71 a	19.3% ± 0.62 a	21.2% ± 0.31 c
Irrigation -75%	0.0% ± 0.00 a	1.8% ± 0.16 a	6.9% ± 0.56 a	9.4% ± 0.21 b	9.5% ± 0.61 b	13.1% ± 1.17 a	14.2% ± 0.71 b	19.3% ± 0.78 c
Factor (*p*-value)	n.s.	n.s.	0.121	0.003	0.010	n.s.	<0.001	0.005

Data are expressed as the mean ± standard error (n = 5). Different letters in the same column indicate statistically significant differences between treatments according to Tukey's post-hoc test ($\alpha = 0.05$). n.s.: non-significant at ◎ = 0.05.

Considering the assessment of the water status of the trees, the water potential was determined, and the results are displayed in Figure 2. According to the FAO calculation method for the estimation of crop water requirement [35], the crop evapotranspiration (ETc) including rainfalls was 109.4 mm, which was slightly higher than the irrigation applied in the control trees (92 mm). As observed in Figure 2, the irrigation control allowed to keep Ψstem MD higher than -2 MPa, except in the record of September (245 DOY <2.25 MPa). In the same way, the treatment involving -60% deficit irrigation entered water stress at two points between July and September, whereas -75% deficit irrigation remained under water stress for the whole season.

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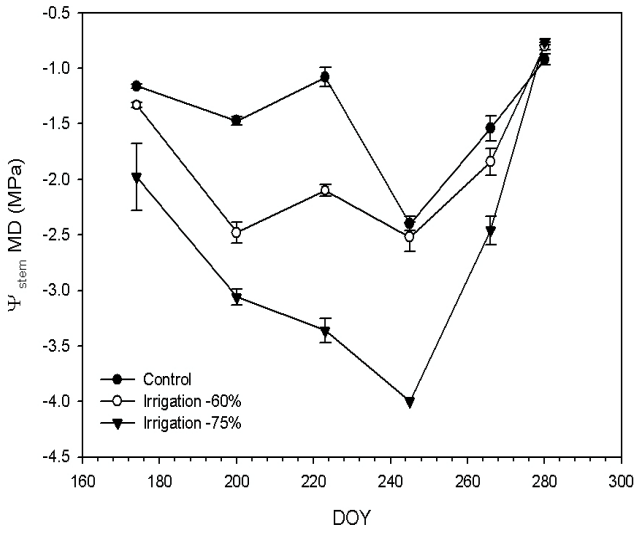

Figure 2. Water potential measurements of deficit irrigation treatments from the fruit set to pre-harvest time. Data are expressed as the mean ± standard error (n = 5).

According to the present results, the treatments involving the -66% thinning and -75% irrigation were involved with the control for the subsequent analyses, representing the most severe conditions for each agronomical technique. Three critical sampling dates were considered, including Sept. 2nd, Sept. 23rd, and Nov. 15th, coinciding with the final harvest time. As shown in Table 2, trees with low fruit load (Thinning -66%) had a short-term significant increase in olive fresh and dry weight, according to the first sampling date. In contrast, both parameters remained similar to the control over fruit ripening, with the exemption of dry weight, which was significantly increased at the end of the experiment. In contrast, the deficit irrigation practice had a significant negative effect on fresh and dry weight throughout the experiment. Concerning the pulp: stone ratio, subtle differences were observed between the control and both practices since only the deficit irrigation significantly reduced the ratio at the end of the experiment. Conversely, both techniques significantly increased the maturity index of fruits, although no significant differences were reported at the end of the experiment. In productive terms (Table 2), the production (kg/plant) of -75% deficit irrigation treatment decreased by 80% compared to the control. Meanwhile, thinning resulted in a halving of production compared to the control. Furthermore, both the water deficit and the thinning treatments reduced the plant's fruit load; consequently, due to the larger availability of primary metabolites, the ripening proceeded faster than in control. Indeed, the maturity index is higher in the deficit irrigation thesis on the first and second sampling dates (Table 2).

Table 2. Olive fruit quality and production parameters at selected sampling dates for -66% thinning and -75% deficit irrigation treatments.

Sampling Date	Treatments	Fresh Weight (g)	Dry Weight (g)	Pulp:Stone Ratio	Maturity Index
September 2nd	Control	1.11 ± 0.06 ^{b}	0.55 ± 0.03 ^{b}	1.46 ± 0.03 ^{a}	2.1 ± 0.07 ^{c}
	Thinning -66%	1.39 ± 0.05 ^{a}	0.71 ± 0.03 ^{a}	1.07 ± 0.11 ^{b}	2.9 ± 0.04 ^{b}
	Irrigation -75%	0.63 ± 0.05 ^{c}	0.39 ± 0.03 ^{c}	1.01 ± 0.07 ^{b}	4.0 ± 0.07 ^{a}
	p-value	< 0.001	< 0.001	0.003	< 0.001
September 23rd	Control	1.40 ± 0.09 ^{a}	0.69 ± 0.05 ^{a}	1.80 ± 0.05 ^{a}	3.20 ± 0.09 ^{c}
	Thinning -66%	1.48 ± 0.07 ^{a}	0.70 ± 0.05 ^{a}	1.93 ± 0.11 ^{a}	4.50 ± 0.06 ^{b}
	Irrigation -75%	0.90 ± 0.03 ^{b}	0.44 ± 0.01 ^{b}	1.65 ± 0.12 ^{a}	6.00 ± 0.11 ^{a}

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	p-value	< 0.001	< 0.001	n.s.	< 0.001
November 15th	Control	2.46 ± 0.08 a	1.10 ± 0.03 b	3.04 ± 0.08 a	6.90 ± 0.01 a
	Thinning -66%	2.85 ± 0.15 a	1.41 ± 0.12 a	3.47 ± 0.20 a	7.00 ± 0.01 a
	Irrigation -75%	1.89 ± 0.09 b	0.73 ± 0.01 c	1.78 ± 0.05 b	7.00 ± 0.02 a
	p-value	< 0.001	< 0.001	< 0.001	n.s.

Sampling date	**Treatments**	**Canopy volume (m^3/tree)**	**Production (kg/plant)**
November 15th	Control	2.69 ± 0.04 b	1.13 ± 0.15 a
	Thinning -66%	3.15 ± 0.01 a	0.52 ± 0.09 b
	Irrigation -75%	1.97 ± 0.01 c	0.20 ± 0.03 b
	p-value	0.001	< 0.001

Data are expressed as the mean ± standard error (n = 5). Different letters in the same column indicate statistically significant differences between treatments according to Tukey's post-hoc test ($\alpha = 0.05$). n.s.: non-significant at ◎ = 0.05.

3.2. Fruit Load and Deficit Irrigation Effect on the Phenolic Profile of Olive Extracts

The effect of decreased crop load and deficit irrigation was investigated throughout the UHPLC/QTOF-HRMS untargeted metabolomics approach of olive fruits. A total of 233 different phenolic compounds were annotated within all analyzed samples, and they are reported in Table S2, together with their phenolic class and subclass and analytical parameters. From these, 107 compounds of flavonoids were reported, mostly represented by anthocyanins and flavones. Moreover, LMW and other phenolics (58 compounds) were widely represented, mainly including alkylphenols and tyrosols. Interestingly, the class of phenolic acids (43 compounds) contained >80% of hydroxycinnamic acids. Finally, lignans and stilbenes classes showed the lowest contribution, with only 25 compounds (Table S2). These results indicate that olive fruits have a high heterogeneity of polyphenols, being considered a rich source of these compounds.

To investigate the effect of harvest time and agronomical techniques (thinning and deficit irrigation) on the phenolic profile of olive fruits, a multivariate HCA was performed to naively cluster olive extracts according to their similarities ascribed to both factors (Figure 3). The results indicate the impact of harvest time on the phenolic profile of olive fruits, obtaining two separated clusters that grouped t1 and t2 together and apart from t3, which clustered independently. Concerning treatments, they played a differential role in the phenolic profile of olive fruits, showing that deficit irrigation depicted an exclusive profile at all times, whereas thinning was found to promote a highly similar profile to that of the control regardless of the harvest time (Figure 4).

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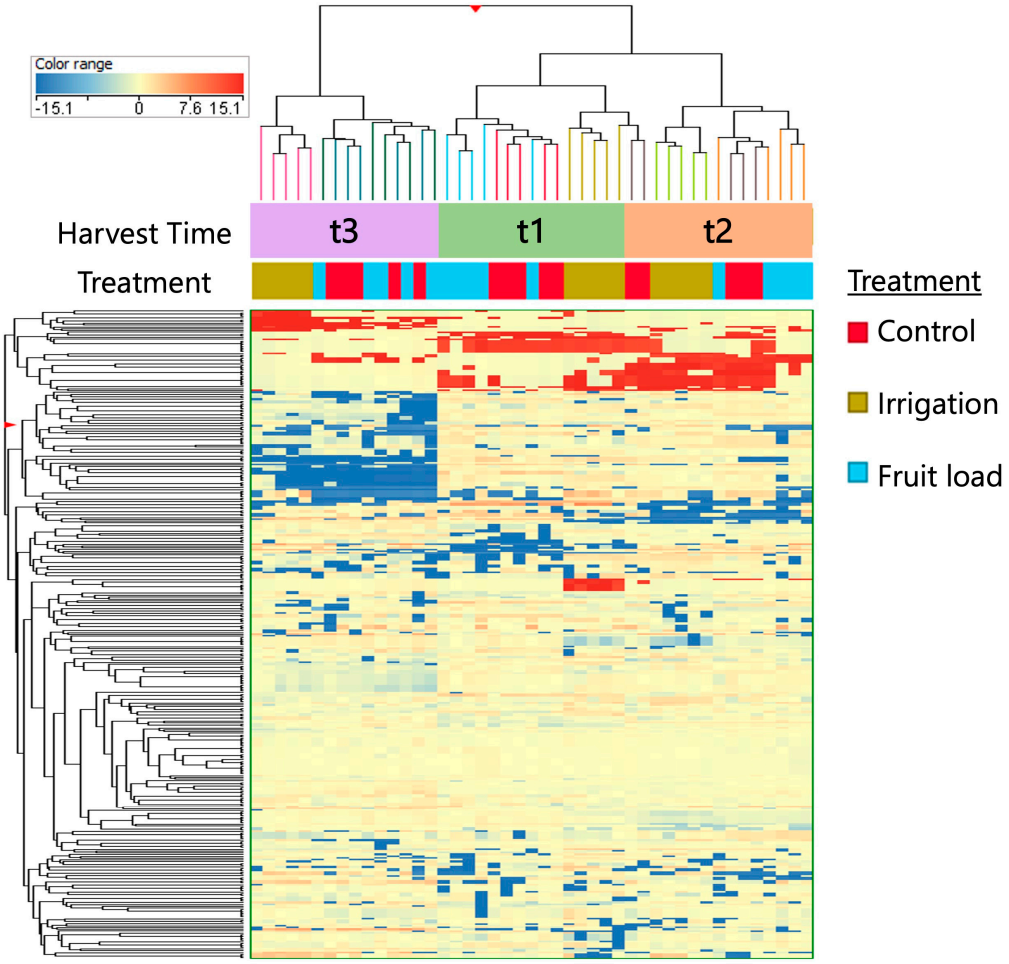

Figure 3. Hierarchical cluster analysis (HCA) on the effect of deficit irrigation and thinning on the phenolic profile of olive fruits (Euclidean distance, Ward's linkage rule).

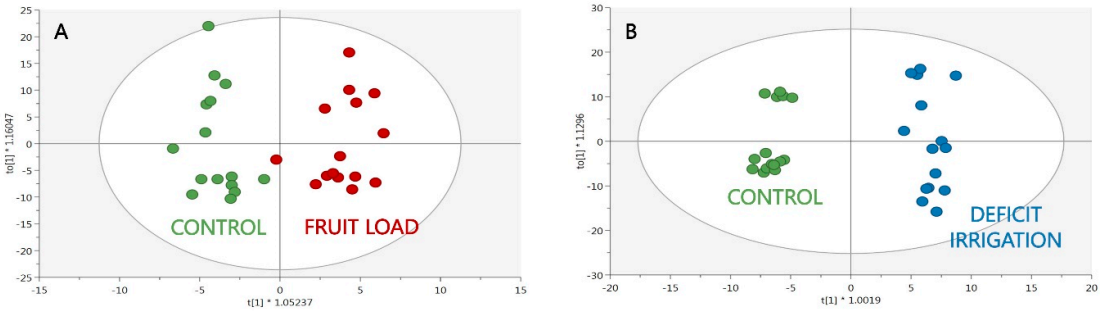

Figure 4. Orthogonal projection to latent structures (OPLS) models were built for the discrimination of thinning (**A**) and deficit irrigation (**B**) according to the phenolic profile of olive fruits.

Once the differential influence of deficit irrigation and thinning on the phenolic profile of olive fruits was assessed over time, a supervised OPLS discriminant analysis (OPLS-DA) was performed to identify those metabolites showing the strongest contribution to the discrimination between each agronomical technique and control conditions: thinning (Figure 4A) and deficit irrigation (Figure 4B). The quality of both models was assessed by the goodness-of-fit (R^2) and goodness-of-prediction parameters (Q2): $R^2 = 0.880$ and $Q^2 = 0.537$ for the thinning-associated model and $R^2 = 0.976$ and $Q^2 = 0.888$ for the deficit irrigation-associated model. The highest quality values observed for deficit

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irrigation may confirm the information provided earlier by HCA, which suggested a distinctive profile associated with this technique for control rather than thinning.

Concerning the OPLS model associated with thinning (Figure 4A), the list of VIP markers (Table 3) shows a predominance of flavonoids, which are found either up- or down-accumulated, thus suggesting a deep modulation of their biosynthesis due to the application of this agronomical technique. Among the most discriminant compounds, flavonoid glycosides are mainly found, except for flavanols, which were found in their aglycone forms. Among them, the anthocyanin glycoside malvidin 3-O-(6''-*p*-coumaroyl-glucoside) accumulated (logFC = 4.0), whereas for spinacetin glycosides a decreased accumulation was reported (logFC = -4.0 – -0.75). This fact also suggests that flavonoid mobilization towards olive fruits is highly influenced by thinning, although there is not a clear differential behavior compared to control. Besides flavonoids, phenolic acids, LMW, and other phenolics were also spotted as VIP markers of thinning in a lower proportion (Table 3). In the case of phenolic acids, in contrast to what was observed for flavonoids, a general accumulation of deglycosylated hydroxycinnamic acids and derivatives, such as 3,4-dicaffeoylquinic acid, sinapine, and ferulaldehyde was reported (logFC = 0.27 – 1.35), together with a repressed accumulation of hydroxycinnamic acid glycosides and esters, represented by 1,2-diferuloylgentiobiose and *p*-coumaric ethyl ester (logFC = -0.28 and -0.15, respectively). A parallel trend was attributed to LMW and other phenolics since free forms of the secoiridoid ligstroside (*p*-HPEA-EDA, logFC = 0.28) and catechols (3-methylcatechol, logFC = 0.38) were found accumulated. In contrast, a decreased accumulation of acetylated derivatives was observed, as reported for tyrosol acetate (*p*-HPEA-AC, logFC = -0.71) and acetyl eugenol (logFC = -0.32).

In contrast, the OPLS model attributed to deficit irrigation (Figure 4B) was combined with a heterogeneous representation of VIP markers, featuring phenolic acids (35%), flavonoids (25%), LMW, and other phenolics (25%; Table 4). Interestingly, all VIP markers associated with deficit irrigation were found accumulated with respect to the control (logFC = 0.07 – 2.33 for all compounds). These results indicate a diversified metabolic response of olive fruits towards deficit irrigation, involving the accumulation of phenolic compounds. Considering the different classes, phenolic acids were exclusively represented by hydroxycinnamic acids, from which those presenting an aglycone form exhibited the highest accumulation rates, i.e.: sinapine, coumaric acid, and 3-sinapoylquinic acid (logFC > 1.0), whereas gentiobioside glycosides exhibited a lower accumulation trend compared to control (logFC < 1.0). In the case of flavonoids, only glycosylated forms were presented among discriminant markers, featuring the chalcone phloretin 2'-O-xylosyl-glucoside (logFC = 1.65), whereas the rest of the flavonoid glycosides showed a little contribution (logFC = 0.26 – 0.51; Table 4). Among LMW and other phenols, ligstroside presented a marked accumulation (logFC = 1.42), whereas other compounds like the coumarin scopoletin and pyrogallol were found also accumulated to a lesser extent (logFC = 0.75 and 0.71, respectively).

Table 1. List of VIP (Variable Importance in Projection) markers, associated with the OPLS-DA model of the untargeted phenolic profile attributed to thinning in olive extracts.

Phenolic class	Phenolic Subclass	Compound Name	VIP Score[1]	LogFC[2]
Flavonoids	Flavones	Apigenin 6,8-di-*C*-glucoside	2.31 ± 1.54	-0.32
	Chalcones	Butein	1.68 ± 0.79	1.12
	Flavanones	6-Prenylnaringenin	1.65 ± 1.82	-0.38
	Flavones	Luteolin 7-*O*-glucuronide	1.63 ± 1.93	0.34
	Anthocyanins	Malvidin 3-*O*-(6''-*p*-coumaroyl-glucoside)	1.62 ± 1.20	4.00
	Isoflavonoids	Glycitin	1.59 ± 2.67	0.68
	Flavones	Isorhoifolin	1.55 ± 0.84	-0.28

 doi:10.20944/preprints202404.1440.v1

	Flavonols	Spinacetin 3-*O*-(2""-*p*-coumaroylglucosyl) (1-6) -[apiosyl(1-2)]-glucoside	1.44 ± 1.95	-0.75
	Flavones	5,6-Dihydroxy-7,8,3',4'-tetramethoxyflavone	1.42 ± 0.87	1.11
	Anthocyanins	Cyanidin 3-*O*-rutinoside	1.41 ± 1.32	-0.21
	Flavonols	Spinacetin 3-*O*-glucosyl-(1-6) -[apiosyl(1-2)]-glucoside	1.37 ± 1.38	-4.00
	Flavanols	Theaflavin	1.35 ± 0.81	-0.63
	Flavanols	(+)-Catechin	1.35 ± 1.49	0.13
Lignans	Lignans	Conidendrin	1.50 ± 1.12	0.36
	Lignans	Secoisolariciresinol	1.41 ± 1.34	-0.52
LMW and others	Hydroxycinnamaldehydes	Ferulaldehyde	1.64 ± 1.07	0.27
	Alkylphenols	3-Methylcatechol	1.41 ± 1.08	0.38
	Hydroxyphenylpropenes	Acetyl eugenol	1.35 ± 1.36	-0.32
	Tyrosols	p-HPEA-EDA	1.34 ± 1.04	0.28
	Tyrosols	p-HPEA-AC	1.32 ± 1.84	-0.71
	Other polyphenols	Coumestrol	1.30 ± 1.49	-4.00
Phenolic acids	Hydroxycinnamic acids	Sinapine	1.92 ± 2.32	1.35
	Hydroxybenzoic acids	Ellagic acid	1.58 ± 1.15	0.80
	Hydroxycinnamic acids	3,4-Dicaffeoylquinic acid	1.50 ± 1.54	1.18
	Hydroxycinnamic acids	*p*-Coumaric acid ethyl ester	1.43 ± 1.22	-0.15
	Hydroxycinnamic acids	1,2-Diferuloylgentiobiose	1.38 ± 1.10	-0.28
Stilbenes	Stilbenes	d-Viniferin	1.76 ± 1.17	0.80

1VIP score was expressed as the mean ± standard error provided by CV-ANOVA validation (n = 6). 2Logarithm of fold change, expressed as the log2-transformed abundances of VIP markers calculated for the pairwise comparison F vs. Control.

Table 2. List of VIP (Variable Importance in Projection) markers, associated with the OPLS-DA model of the untargeted phenolic profile attributed to deficit irrigation in olive extracts.

Phenolic Class	Phenolic Subclass	Compound Name	VIP Score[1]	LogFC[2]
Flavonoids	Dihydrochalcones	Phloretin 2'-*O*-xylosyl-glucoside	1.60 ± 0.54	1.65
	Flavanones	Naringenin 7-*O*-glucoside	1.58 ± 0.51	0.48
	Anthocyanins	Petunidin 3-*O*-rhamnoside	1.41 ± 0.38	0.27
	Flavones	Apigenin 6,8-di-*C*-glucoside	1.40 ± 0.26	0.07
	Flavonols	Myricetin 3-*O*-rhamnoside	1.39 ± 0.57	0.15
Lignans	Lignans	Cyclolariciresinol	1.63 ± 0.35	1.38
	Lignans	Secoisolariciresinol	1.56 ± 0.41	0.20
	Lignans	Sesamol	1.42 ± 0.67	1.09
LMW and others	Other polyphenols	Pyrogallol	1.54 ± 0.63	0.72
	Curcuminoids	Curcumin	1.53 ± 0.73	0.55

 doi:10.20944/preprints202404.1440.v1

	Tyrosols	Ligstroside	1.52 ± 0.33	1.42
	Hydroxycoumarins	Scopoletin	1.47 ± 0.56	0.75
	Other polyphenols	3,4-Dihydroxyphenylglycol	1.38 ± 0.63	0.48
Phenolic acids	Hydroxycinnamic acids	Cinnamic acid	1.66 ± 0.74	0.94
	Hydroxycinnamic acids	Sinapine	1.65 ± 0.76	2.33
	Hydroxycinnamic acids	Caffeoyl aspartic acid	1.60 ± 0.50	0.79
	Hydroxycinnamic acids	3-Sinapoylquinic acid	1.59 ± 0.42	1.25
	Hydroxycinnamic acids	*p*-Coumaric acid	1.44 ± 1.01	1.92
	Hydroxycinnamic acids	1-Sinapoyl-2-feruloylgentiobiose	1.38 ± 0.52	0.68
	Hydroxycinnamic acids	1,2-Diferuloylgentiobiose	1.37 ± 1.09	0.60

1 VIP score was expressed as the mean ± standard error provided by CV-ANOVA validation (n = 6). 2Logarithm of fold change, expressed as the log2-transformed abundances of VIP markers calculated for the pairwise comparison Deficit Irrigation vs. Control.

Finally, to shed light on the absolute effect of the two agronomical techniques tested in this work, a semi-quantification of phenolic classes was performed in olive extracts, and the results are depicted in Figure 5. Regarding the different phenolic subfamilies, LMW phenolics achieved the highest content rates, with maximum contents being >20 mg g^{-1}FW, followed by far by phenolic acids and lignans, reaching content peaks of 1 – 2 mg g^{-1}FW, whereas all flavonoid subclasses presented a content < 1 mg g^{-1}FW and stilbenes reflected the lowest contents (< 0.5 mg g^{-1}FW). Referring to the factors involved in this study, i.e.: time and agronomical techniques, both played a significant role in the phenolic content of olive fruits.

Thus, in the case of LMW and other phenolics (Figure 5G), the results indicate that both agronomical techniques significantly increased the content of this subfamily with respect to control in a time-dependent manner ($p < 0.05$): thinning led to a significant increase at a short-term (t1, 23.3 mg g^{-1}FW), whereas deficit irrigation promoted a significant increase at a mid-term (t2, 24.4 mg g^{-1}FW) and the content was harshly decreased at long term regardless of the treatment (t3, 3.33 – 7.77 mg g^{-1}FW for all treatments). A similar trend was observed for lignans (Figure 5E), as deficit irrigation promoted a mid-term significant increase (t2, 1.11 mg g^{-1}FW), whereas a marked decrease was reported after long harvest times (t3, < 0.77 mg g^{-1}FW for all treatments). Equally, deficit irrigation promoted a sustained significantly increased phenolic acid content up to 1.09 – 1.33 mg g^{-1}FW, reporting 51.1%, 28.4%, and 32.5% improvements with respect to control at t1, t2, and t3, respectively (Figure 5F). Concerning flavonoid subfamilies, the accumulation of flavanols, flavones, and flavonols followed a similar trend: treatments did not induce a substantial effect on their content and a long-term decrease was observed in all cases (Figures 5B, 5C, and 5D, respectively). Finally, anthocyanins and stilbenes contents were not markedly altered by any factor, showing slight variations over time regardless of the treatment (less than 5% variations due to any condition for both subfamilies at all times; Figures 5A and 5H).

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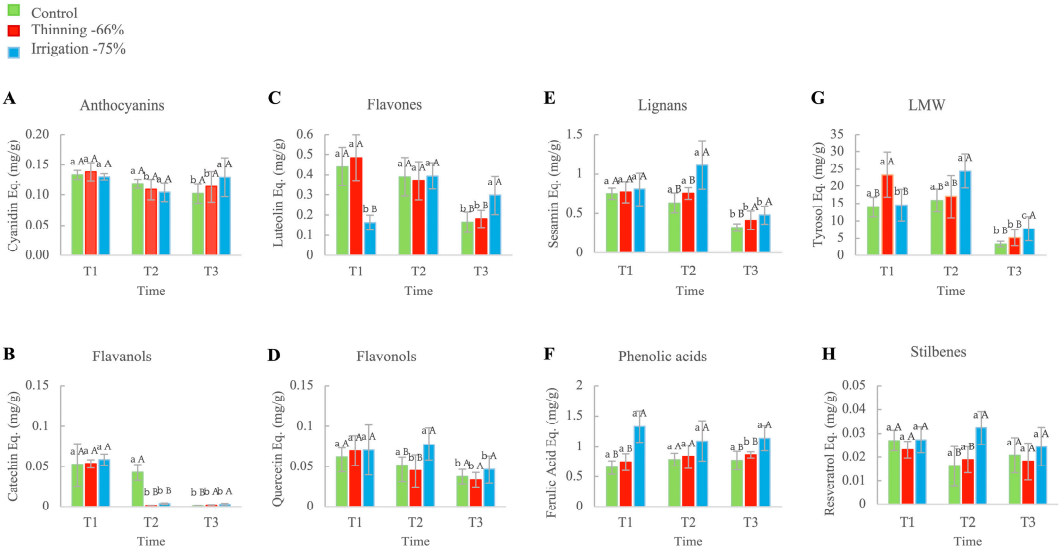

Figure 5. Semi-quantification of each subfamily of phenolic compounds (A-H). All results are expressed in mg g^{-1} of equivalents (Eq.) for each reference compound. Vertical bars indicate the standard deviation (n = 6). Different lowercase letters indicate statistically significant differences ($p < 0.05$) within the same treatment at different sampling times. Different capital letters indicate statistically significant differences ($p < 0.05$) among treatments within the same sampling time.

Overall, the results provided by the untargeted metabolomics approach on the phenolic profile of olive fruits demonstrate that LMW and other phenolics, phenolic acids, and lignans are involved in the response attributed to the agronomical techniques tested. In contrast, flavonoids exhibited differential patterns of accumulation between their forms that do not reflect a quantitative change in accumulation. Especially, deficit irrigation promoted a mid-term enhancement of the accumulation of their content, leading to an exclusive metabolic fingerprinting as reported by the phenolic profile of olive fruits.

4. Discussion

The results in the present study provide important information for modern olive growing, whose goal is to produce Italian high-quality extra virgin olive oil at lower prices, considering an ongoing change in the olive oil sector to reach an efficient and sustainable productive system, with the ability to respond to current threads associated with climate change. In this perspective, high-density olive groves, designed to increase and make production more consistent, face the challenge of reducing inputs and obtaining a product with enhanced health-promoting properties and quality. Therefore, from the agronomic point of view, we tested the effect of fruit load and deficit irrigation on oil accumulation and fruit ripening of the Sikitita cultivar.

Fruit load is one of the main carbon sinks on the tree and it can influence primary metabolism by affecting carbon partitioning. In the case of high fruit loads, the vegetative growth is reduced, and a condition of carbohydrate scarcity is also induced, causing low fruit quality at harvest [41]. In our experiment fruit load was varied by applying flower thinning that allowed to delete the fruit carbon uptake before they became a significant sink for the tree. Fruit load affects the level of carbon demand without changing the carbon supply potential of the tree. Therefore, low fruit load reduces carbon uptake demand.

In our study, a lighter fruit load led to an intermediate oil accumulation in fruits of intermediate thinning trees (-33% and -50%), whereas the fresh and dry weight of fruits increased with the most intense conditions, -66% (Table 2). However, thinning caused a significant reduction in fruit yield per plant. In this context, Lavee & Wodner, 2004 [42], suggested that fruits of comparable size from both

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high-yielding and low-yielding trees have equivalent metabolic capabilities for oil synthesis. It also showed in Barnea and Manzanillo cultivars that the relative amount of oil in the fruit, once reaching an annual maximum, is not fruit size dependent, but it is based on genetic and environmental conditions. However, there is a general assumption by several authors confirming that the accumulation and concentration of oil within the fruit are significantly reduced with high crop loads **[15,18]**. The different responses of fruit oil content between these studies likely depend on the source:sink balance for carbon in olive trees during fruit development [14].

Irrigation has been shown to negatively affect both oil yield and quality, reporting that oil in the fruit decreases as the amount of water applied increases [43]. However, water deficit significantly affects the availability of carbohydrates by reducing leaf photosynthesis, mostly due to stomatal restrictions, as well as the formation of new leaves over the season [44].

Our results showed that deficit irrigation applied in this study resulted in a significant oil yield loss in the fruit and production per plant. This is attributable to the achieved values of water potential (Figure 2). This finding is consistent with previous reports, where oil content in the mesocarp decreased as the tree's water deficit level increased [14]. Indeed, identified values between -2.0 and -2.5 MPa as the threshold for the onset of water stress, significantly impacting oil yield and quality [45]. In contrast, the trial by Marra et al., 2016 [46], in olive groves of cv. Arbequina showed how a return of 50-60% of the Crop Irrigation Requirement, corresponding to a water potential value of about -3 MPa, and a seasonal irrigation volume of 130 mm, resulted in maximum production while maintaining high product quality.

In our experiment, light fruit load and water deficit caused a decrease in yield, leading to faster ripening over time. These results are in accordance with previous reports indicating that low fruit load leads to precocious olive veraison (i.e., color change), which is generally associated with the advancement of the ripening process [47]. This clearly indicates that the availability and/or the concentration of primary metabolites can have a significant influence on the onset of the ripening process [33]. This assumption has been demonstrated in other species such as grapevine, where the veraison process and the concomitant accumulation of phenolic compounds (i.e., synthesis of anthocyanins) are correlated with the concentration of carbohydrates in the fruit [48].

Afterward, an untargeted metabolomics approach was performed to decipher the effect of thinning and deficit irrigation overtime on the chemical composition of olives. As previously discussed, the harvest period played a key role in plant productivity (oil in the fruit) and it also impacted the phenolic composition of olives, suggesting an important contribution of these metabolites in the management of the applied agronomical techniques. Hence, optimizing olive harvesting becomes a critical stage to ensure the highest amount of oil of a predefined level of quality [49]. The application of unsupervised multivariate statistics on the phenolic profile of olive extracts, through hierarchical cluster analysis, showed that harvest time had a significant impact on the phenolic profile of olives (Figure 3). The t3, which corresponds to the olive's full maturation stage, clustered independently from t1 and t2, the mesocarp development stage and the green mature stage, respectively. These results are in line with those by Karagiannis et al., 2021 [50], who reported a unique profile of secondary metabolites associated with the black mature stage, the full ripening of the olive, being independent of the other maturation stages.

Regarding treatments, the phenolic compound profile was affected by both water deficit and crop load, but the first explained a larger effect. Indeed, this is not surprising since fruit load mainly alters the availability of primary metabolites, while water deficit induces a complex response by the plant that involves many other physiological pathways such as water regulation and drought-escaping mechanisms [51]. Indeed, phenolic compounds are usually involved in stress response, in our study they resulted in sensitivity, though to a lesser extent to fruit load too. In thinning treatment, the increase in phenolics (particularly LMW phenolics, including secoiridoids), was more consistent during the early phases of the ripening (t1, Figure 5G). Similar results were obtained in the study by Ivancic et al., 2022 [52], where the total polyphenol content increased during the ripening process until it reached a maximum and then decreased. In the same study, the highest polyphenol content was observed in the olives' skin at the last stage of ripening due to the accumulation of anthocyanins.

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In our study, a clear accumulation of the anthocyanin glycoside malvidin 3-O-(6"-*p*-coumaroyl-glucoside) was observed in the case of thinning. Similarly, in peaches [53] crop load did not influence the content of phenolics, except anthocyanins, the only group affected by thinning. In another study by Andreotti et al., 2010 [54], on nectarine fruit, total phenolic compounds were influenced by fruit load. The accumulation of flavanols and cinnamic acids was significant with fruit thinning. Those findings are in accordance with the present results, where a general accumulation of hydroxycinnamic acids and derivatives was found due to thinning. Interestingly, a decreased accumulation of acetylated phenolic acids was observed, suggesting an increased susceptibility of this phenolic subclass to oxidation [55] which can be motivated by the induction of mild stress conditions associated with this agronomical technique.

Regarding water deficit, our results revealed that all VIP markers increased compared with the control. Among discriminant markers for deficit irrigation treatment, all flavonoids were found under their glycosylated, in agreement with previous results, where a specific accumulation of the flavonoid glycosides, such as luteolin 7-O-glucoside, apigenin 7-O-glucoside, and quercetin-3,7′-di-O-glucoside was generally observed **[45,51]**. Nevertheless, contrasting results also indicate that deficit irrigation did not play a significant impact on the distribution of phenolic compounds and total polyphenol content of olive oil [31]. Thus, prompting the development of further studies to accurately elucidate the involvement of flavonoids in the response to deficit irrigation. In our study, besides flavonoids, hydroxycinnamic acids were found to greatly accumulated due to this agronomical technique. In this case, there is a general assumption that water stress induces an increase in hydroxycinnamic acid amides as an antioxidant mechanism to resist the overproduction of reactive oxygen species (ROS) under these conditions [56]. In parallel, the deficit irrigation-mediated accumulation of hydroxycinnamic acids could be a consequence of the reduction in lignin biosynthesis, as they are recognized precursors of lignin, and it has been suggested that the decrease in lignification is an adaptive response to water stress [57]. Furthermore, in our experiment, concerning LMW and other phenols, ligstroside showed a marked accumulation due to deficit irrigation. Ligstroside is one of the most important secoiridoids of olive fruit, and the concentration of this compound increases with fruit development [58]. Anyway, in agreement with our findings, Machado et al., 2013 [59], reported that secoiridoids derivatives, including the 3,4-dihydroxyphenylethyl alcohol-deacetoxyelenolic acid dialdehyde (3,4-DHPEA-EDA), oleuropein and ligstroside, decreased as olive and water availability increase. Similarly, Cirilli et al., 2017 [60], reported that phenolic contents were found to increase under rainfed conditions, also indicating that phenolic catabolism was particularly modulated by water availability during the early stages of fruit development. In our case, the deficit promoted a significant increase at a mid-term for LMW and significantly increased phenolic acids.

The present study did not consider the processing stage since it wanted to evaluate the variability in the chemical composition of the olive fruit derived from the agronomic techniques applied, as the extraction method would further affect the phenolic content of the resulting oil differently. Furthermore, in general terms, only a small percentage of total phenols present in the fruits was transferred to the oils for all cultivars (0.38%-1.95%). Specifically, in cultivar Sikitita, only 0.67% of phenolic content remained in the oil. Among all phenolic groups, secoiridoids were the compounds with the highest transfer rate from fruits to oil, followed by flavonoids and simple phenols [61].

5. Conclusions

Water deficit had a much more significant effect on fruit growth dynamics, maturation index, and oil yield than fruit load. Based on the reported results, the effect of fruit load on oil yield and quality can be mitigated by choosing the optimal ripening period for harvest. Although the oil yield is highest at the later harvest time, the accumulation of most phenolic classes decreases over the long term. Fruit chemical composition was strongly influenced by fruit load, deficit irrigation, and harvest time, as indicated by the phenolic profile of olive extracts. A distinctive metabolic response on the phenolic profile of olives was observed, suggesting unique re-programming fates associated with

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these agricultural techniques. In general, LMW phenolics, essentially represented by tyrosols and hydroxycinnamic acids, were involved in managing the response to both treatments. Flavonoids, on the other hand, showed different responses among their forms: a more specific response in the case of fruit load, guided by flavonoid glycosides, and a broader response in the case of irrigation, concerning flavonoid aglycones. Based on these data, it was possible to discern the effect of environmental and carbon partitioning phenomena on the promotion of specific classes of key secondary metabolites. Further research should be aimed to get a clear insight into the effect of agronomic practices on both production parameters and the modulation of phenolic metabolism, particularly how primary metabolites and/or environment regulate physiological pathways. For this purpose, a multi-year experimental design would be highly recommended. Overall, the approach described here sheds light on the functional characterization of two agronomical techniques, paving the road to establishing novel and efficient practices leading to a more profitable and sustainable system for olive oil production.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: Data on the vegetative and productive situation of olive trees in full bloom (May 21st, 2021) and pre-harvest time (October 7th, 2021). The data present shoot length (cm), shoot nodes (number/shoot), number of inflorescences and fruits (number/shoot), current year apical shoot length (cm), and number of nodes present on the current year shoot (number/apical shoot). Table S2: Dataset on the phenolic profile of olive extracts from untargeted metabolomics. The annotated compounds are provided together with phenolic class and subclass, individual abundance, retention time (min), composite mass spectra (accurate mass and intensity combinations), and molecular formula.

Author Contributions: Conceptualization, C.F. and S.T.; methodology, S.T., L.L. and P.G.P.; software, C.F. and P.G.P.; formal analysis, C.F., S.T. and P.G.P.; investigation, C.F., S.T. and P.G.P.; resources, C.F., S.T., L.L. and P.G.P.; data curation, C.F and P.G.P.; writing—original draft preparation, C.F and P.G.P.; writing—review and editing, C.F., S.T., L.L., E.C and P.G.P..; visualization, C.F. and P.G.P.; supervision, S.T. and E.C.; project administration, C. F., S.T and E.C.; funding acquisition, E.C. and L.L. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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