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Effects of Nitrogen Foliar Fertilization on the Vegetative and Productive Performance of the Olive Tree and on Oil Quality

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Abstract: The correct management (dose, time of distribution) of N fertilization in olive growing is still not completely clarified but is nowadays essential in order to guarantee sustainable production. In this regard, in central Italy over a 4-year-period a study was carried out to investigate the effect of high nitrogen availability during oil accumulation in the fruit (second phase of fruit growth) on vegetative and productive activities of olive trees and oil quality. In May of each year, secondary branches were selected and girdled in their proximal part. Afterwards, half of the girdled branches were sprayed three times with a solution containing urea (2% w/w), whereas the other half was sprayed only with water. The nitrogen treatments did not cause any damage to the foliage and fruits nor did it cause appreciable changes in leaf photosynthesis and specific weight, fruit-drop, ripening pattern and weight, water and oil contents, pulp/pit ratio of the fruits, fatty acid composition, polyphenols content, and sensorial characteristics of the oil. The N provided via foliar fertilization during the oil accumulation phase in trees in conditions of good supply of N does not induce significant effects on the vegetative-productive activity of the tree.

Keywords: *Olea europaea* L.; foliar nitrogen fertilization; N management; oil quality

1. Introduction

Nitrogen is the mineral nutrient most commonly applied in olive (*Olea europaea* L.) orchards since it is a major nutritional factor affecting plant growth [1–3]. N shortage results in a marked decrease in plant photosynthesis since a great part of the total leaf N is allocated to the photosynthetic apparatus [4].

N deficiency, therefore, affects, to various extents, sugar metabolism and/or carbohydrate partitioning between source and sink tissues [5–7]. Low N availability causes reduction in leaf N, reduced number of flowers per inflorescence, low fruit set and yield [8–11]. N fertilization was also reported to increase fruit set in olive [8]. Among the possible methods for fertilization, there is foliar fertilization, which is one of the most commonly used in olive groves. In particular, combining soil and foliar urea application was seen to be more effective in increasing leaf N than only soil application [12]. Multiple application of urea (2%) via foliar spray to olive trees with inadequate nitrogen status increased leaf N significantly but did not affect the percentage of flowering nodes or flower size at anthesis in May [13].

Since the relative low cost of N fertilization, olive growers have increased the amount of N fertilizers used based on the perception that this can increase yields and so N is usually supplied in excess [3]. Some studies, however, have demonstrated no increase in crop growth or olive yield by

N-sufficient trees following N fertilization [3,11,14,15]. At present, many olive orchards are being over-fertilized with nitrogen, causing numerous problems in both the tree and the soil [12]. Moreover, excess fertilizer application is expensive and leads to N losses by leaching with negative impacts on the environment [4]. A correct management (dose, time of distribution) of N fertilization has not been completely clarified yet, but it is nowadays essential in order to guarantee sustainable production as it allows the design of management strategies to reduce the environmental impact of nitrogen losses and the negative effect of excess nitrogen on the tree, and to guarantee optimal tree growth and productivity. At the same time, there is still a lack of knowledge regarding the correct management of N and time guidelines for N fertilization of olive orchards are few [16–18].

Erel et al. [8] reported that the oil phenolic content decreased by increasing leaf N, indicating protein-phenol competition in leaves. On the other hand, polyunsaturated fatty acids (PUFAs) C18:3 increased in response to higher doses of N [9].

The aim of the present study was to investigate the effects of N foliar fertilization on the vegetative and productive activity of the olive tree and on the quality of the oil. In particular, the effects of the N foliar fertilization on the quality of the oil are of great interest because there is still little knowledge in this regard.

2. Material and Methods

2.1. Olive Grove and Environmental Characteristics

The trial was carried out for four years in Central Italy in a 12-year-old rainfed olive grove near Assisi (12°56′ E longitude, 43°11′ N latitude, about 400 m a.s.l.). The trees belong to the Frantoio cultivar, were trained to the vase system, and spaced 6 × 6 m. The soil was managed by green cover mowing.

The climatic data were monitored by a meteorological station near the olive grove and are reported in Table S1. The mean annual precipitation in the area during the years of experimentation was about 900 mm, the minimum temperature (−0.3 °C) was recorded in February, while the highest temperature (32.4 °C) was recorded in July (Table S1). The soil, derived from calcareous marl and classified as Typic Haploxerept (Soil Survey Staff, 2010), is characterised by an alkaline pH in water (1:2.5 soil:water ratio) (8.1 ± 0.3), and a loam texture (sand 41%, silt 34%, clay 25%). The total organic carbon (TOC) was 9.3 ± 0.3 g kg $^{-1}$, total N was 0.7 ± 0.1 g kg $^{-1}$, available P was 8.0 ± 1.1 mg kg $^{-1}$ and exchangeable K was 114 ± 11.0 mg kg $^{-1}$. The olive trees were subjected to the usual cultivation cures and to the ordinary spring fertilization to the soil. In particular, at the beginning of each March, 150 kg ha $^{-1}$ of N as urea and 100 kg ha $^{-1}$ of K as potassium sulphate were distributed on the soil over the green cover without burying.

2.2. Foliar Fertilization

Every year on 10 olive trees homogeneous for vegetative aspect and olive production (about 15 kg of olives per tree), 6 secondary branches per tree were selected. Each May these branches were girdled at the base by removing a ring of bark about 1 cm high in order to interrupt the phloem continuity, to prevent the outflow of the lymph processed by the same branch, and thus localize and amplify in it the effect of the next foliar fertilization. On these trees, during the period of oil accumulation in the drupes (at the end of July, mid-August and mid-September), three of the girdled branches in each tree were subjected to urea foliar fertilization. The spraying was performed mid-morning with handle atomizer, until incipient drip, with a water solution of urea (2% w/w) + wetting agent (Dioctyl Sodium Sulfosuccinate 50 mL/1000 L) (treated). The other three branches per tree were treated with water and wetting agent (control). During the treatment, the branches were isolated from the rest of the canopy and from the soil by a plastic film.

2.3. Leaf N Content

One-year-old fully expanded leaves (three per branch) and current season fully expanded leaves (three per branch) were randomly harvested, a week after the last treatment, from thirty branches in each treatment and combined into a composite sample, weighed and analyzed. Leaf tissue nitrogen (N) was determined in triplicate by the Kjeldahl method.

2.4. Vegetative Activity

On both the treated and control branches the following activities were determined: the growth and the number of nodes of the shoots (measuring the length of the shoots, at the end of the autumn growth on 5 secondary branches per branch). The phytotoxic effect of the treatments was monitored by visual controls.

2.5. Leaf Net Photosynthesis (Pn) and Area Dry Mass (ADM)

Leaf net photosynthesis (Pn) was determined in the morning (from 9:00 to 11:00) on cloudless days on 15 expanded current-season and one-year-old leaves per treatment, randomly sampled from well-lit branches' portions 10 days after each treatment, when the incoming photosynthetic photon flux density (PPFD) was 2056, 2036 and 1990 mmol m-2 s-1 respectively—which is over the light saturation point in olive [19–21]. Pn was determined using a portable ADC LCA-3 gas exchange analyser (Analytical Development Company Ltd., Hoddesdon, UK) and a Parkinson-type assimilation chamber as described by [20]. After the gas exchange measurements, the leaves were immediately transferred to the laboratory in a portable refrigerator for determination of area dry mass (ADM) as described in [22].

2.6. Fruits Production, Maturation Indexes and Oil Characteristics

The number of fruits per inflorescence after the first N treatment and at the harvest was evaluated on six secondary branches per tree. The fruit drop was calculated as a difference between the number of fruit after the first treatment and at harvest on 200 inflorescence per branch. At harvest time, olive production per branch was determined. On 50 olives per tree the following aspects were determined: detachment force, flesh firmness, pigmentation, fresh weight (FW) and dry weight (DW) of the olives (the latter determined by drying the olives at 90 °C), pulp/pit ratio.

The detachment force was measured by a "Carpano et Pons" dynamometer. Flesh firmness was assessed using a "Carpo" hand dynamometer ("Effe.gi" dynamometer DT 05, Alfonsine, Ravenna, Italy) with a 1.0 mm diameter tip. The "Jean pigmentation index", ranging from 0 to 5 with the 0 value for green olives and the 5 value for olives with deep purple pigmentation of the pulp respectively, was visually assessed. The oil and water content of the olives was determined, after fruits crashing, using the "SpectraAlyzer ZEUTEC"—NIR: Near Infra-Red". Each year, one day after harvesting (beginning of November), oil was extracted from two samples of about 2.5 kg per treatment using an artisanal mini olive-mill [23,24]. The determination of the oil chemical characteristics (acidity, peroxide number, total phenol content and fatty acid composition) and the sensory evaluation (panel test) were carried out on 3 subsamples for each oil sample following the Official Methods of Analysis [25].

2.7. Statistical Analysis

All data were analyzed by one-way analysis of variance (ANOVA). Significant differences were assessed by means of the Tukey's honest significant difference (HSD) test at $P = 0.05$. Statistical tests were performed using Graph Pad Prism 6.03 software for Windows (La Jolla, CA, USA).

3. Results

3.1. N Content

The N content of the one-year-old fully expanded leaves one week after the last treatment was rather high both in the treated leaves and in the control ones, settling on values around 1.7% ± 0.34% of dry matter for the treated leaves and 1.3% ± 0.45% for the control. On the other hand, the N content of the current season leaves was higher in the treated leaves (2.1% ± 0.25%) than in control leaves (1.4% ± 0.32%).

3.2. Vegetative Activity

The shoots growth, measured at the end of the autumn, was quite limited both in the control and in the treated branches. However, in the treated branches an elongation greater than in the control branches was observed (1.26 ± 0.31 cm and 0.76 ± 0.20 cm, respectively). The foliar fertilization with urea did not cause visible damage to the leaves.

3.3. Leaf Net Photosynthesis (Pn) and Area Dry Mass (ADM)

The photosynthetic activity of young and one-year-old leaves was not affected by the foliar N treatment. In general, the photosynthetic activity of young leaves was not different from that of one-year-old leaves (Table 1). The specific weight of the leaves in treated branches was not influenced by the treatment nor by the age of the leaves (Table 1).

Table 1. Area dry mass (ADM) and photosynthesis (Pn) in current season and one year old leaves at 10 days after each treatment (DAT).

Leaf Age	Treatment	ADM (mg cm $^{-2}$)	Pn (μmoli CO_2 m $^{-2}$ s $^{-1}$)	ADM (mg cm $^{-2}$)	Pn (μmoli CO_2 m $^{-2}$ s $^{-1}$)	ADM (mg cm $^{-2}$)	Pn (μmoli CO_2 m $^{-2}$ s $^{-1}$)
		10 DAT1		10 DAT2		10 DAT3	
Year 1							
Current season	Control	23.45 b	8.23 a	20.83 a	3.20 a	19.46 a	7.19 a
	Treated	25.04 b	9.26 a	19.85 a	2.84 a	19.90 a	6.85 a
One year old	Control	27.76 a	7.76 a	20.72 a	2.47 a	20.03 a	8.71 a
	Treated	27.16 a	9.15 b	21.45 a	3.05 a	20.01 a	8.41 a
Year 2							
Current season	Control	24.76 a	11.03 a	19.87 a	3.45 a	22.46 a	11.46 a
	Treated	25.10 a	10.87 a	19.90 a	3.56 a	22.90 a	11.35 a
One year old	Control	27.77 a	7.94 a	19.93 a	2.87 a	25.83 a	8.84 a
	Treated	28.43 a	8.38 a	20.05 a	2.95 a	24.03 a	8.17 a
Year 3							
Current season	Control	18.94 a	8.65 a	19.84 a	2.80 a	21.36 a	12.85 a
	Treated	18.55 a	10.31 b	20.55 a	3.75 a	21.80 a	11.75 a
One year old	Control	20.91 a	9.30 b	21.91 a	2.59 a	24.73 a	11.70 b
	Treated	20.34 a	5.65 a	21.35 a	3.15 a	23.93 a	10.09 a
Year 4							
Current season	Control	19.46 a	13.67 a	20.14 a	3.82 a	20.36 a	7.19 a
	Treated	19.90 a	13.07 a	19.75 a	4.45 a	21.09 a	6.85 a
One year old	Control	20.03 a	10.76 a	20.63 a	3.78 a	22.73 a	8.71 a
	Treated	20.01 a	11.41 a	21.75 a	3.49 a	22.93 a	8.41 a

For each year mean values followed by different letters are significantly different ($P < 0.05$) ($n = 15$).

3.4. Fruits Production, Maturation Indexes and Oil Characteristics

The number of fruits per inflorescence at the time of the first treatment and at harvesting time, was not influenced by foliar fertilization in the four years of experimentation. In particular, the fruit set percentage was equal to 2.2% ± 0.4% in the treated branches and 2.0% ± 0.5% in the control branches

Therefore, it can be deduced that the fruit-drop too, which occurred from the time of the first treatment to the harvest, was not modified by foliar fertilization.

The fresh and dry weight of the fruits and the pulp/pit ratio were not significantly different among the fruits from treated and control branches. The water content of the fruit at harvesting time was around 55% in the first year, 47% in the second year, 45% in the third year and 61% in the last year, while the and oil content was around 20% of the fresh weight: they did not change as a consequence of the treatment (Table 2). The lack of effect on the oil content of the fruit suggests that, under normal nutritional conditions, the accumulation of oil in the fruit is not influenced by the high availability of N.

Table 2. Fruits characteristics.

Treatment	Water Content (%)	Dry Weight (DW) (g)	Oil Content (% FW)	Pulp/Pit (FW/FW)	Detachment Force (N)	Colour (0–5)	Pulp Firmness (kg)
			Year 1				
Control	55.0 a	0.85 a	21.57 a	2.71 a	2.63 a	2.22 a	0.32 a
Treated	55.7 a	0.89 a	20.72 a	2.75 a	2.77 a	1.94 a	0.34 a
			Year 2				
Control	46.6 a	1.03 a	22.85 a	3.71 a	3.16 a	2.11 a	0.36 a
Treated	47.2 a	1.05 a	22.59 a	3.68 a	3.00 a	2.19 a	0.34 a
			Year 3				
Control	45.4 a	0.94 a	22.92 a	2.79 a	2.56 a	4.05 a	0.32 a
Treated	46.7 a	0.98 a	21.83 a	2.86 a	2.28 a	3.75 a	0.34 a
			Year 4				
Control	60.8 a	0.64 a	19.91 a	1.79 a	3.45 a	2.61 a	0.52 a
Treated	61.2 a	0.68 a	18.81 a	1.98 a	3.39 a	2.79 a	0.48 a

For each year mean values followed by different letters are significantly different ($P < 0.05$) ($n = 500$).

The course of the maturation of the olives, determined on the basis of their resistance to detachment, the drop, the pigmentation and the hardness of the pulp, was not influenced by foliar fertilization. The quality of the oil was not affected by the treatment either and was excellent in terms of acidity, number of peroxides, total polyphenol content and panel test evaluation (Table 3).

Table 3. Chemical-sensory oil characteristics.

Treatment	Acidity (%)	Peroxides (meq O_2 kg^{-1})	Polyphenols (mg kg^{-1})	Panel Test (1–9)
		Year 1		
Control	0.41 a	5.00 a	609.27 a	8.0 a
Treated	0.43 a	4.00 a	599.79 a	8.0 a
		Year 2		
Control	0.46 a	5.25 a	639.27 a	8.0 a
Treated	0.51 a	6.5 a	599.89 a	7.8 a
		Year 3		
Control	0.29 a	5.3 a	558.44 a	7.9 a
Treated	0.31 a	5.6 a	588.04 a	7.8 a
		Year 4		
Control	0.25 a	8.0 a	468.45 a	7.9 a
Treated	0.35 a	9.5 a	488.05 a	7.9 a

For each year mean values followed by different letters are significantly different ($P < 0.05$) ($n = 6$).

With reference to the acid composition, foliar fertilization induced an increase in linoleic acid and, for one year only, in oleic acid (Table 4).

Table 4. Oil acidic composition.

Treatment	Oleic (%)	Linoleic (%)	Linolenic (%)	Eicosenoic (%)	Margaric (%)	Palmitic (%)	Palmitoleic (%)	Arachic (%)	Stearic (%)	Margaroleic (%)
					Year 3					
Control	62.97 a	9.41 a	0.58 a	0.19 a	0.04 a	13.89 a	1.36 a	0.26 a	2.19 a	0.01 a
Treated	70.18 a	11.31 a	0.59 a	0.21 a	0.04 a	13.74 a	1.18 a	0.31 a	2.32 a	0.01 a
					Year 4					
Control	76.95 a	6.78 a	0.78 a	0.27 a	0.05 a	11.29 a	0.91 a	0.38 a	2.47 a	0.11 a
Treated	76.08 a	7.33 a	0.72 a	0.26 a	0.06 a	11.94 a	1.14 a	0.31 a	2.05 a	0.09 a

For each year mean values followed by different letters are significantly different ($P < 0.05$) ($n = 6$).

4. Discussion

The leaves' N content was high both in control and treated leaves [12,26]. The absence of substantial differences between the N content in one-year-old treated and control leaves after one week from the last treatment can be attributed both to the modality of urea absorption that occurs mainly due to the young leaves (in which the thick layer of the cuticle is not yet present), and to the rapid translocation of N from the leaves to the sinks present (fruits) [15,27]. Indeed, the N content in the current season leaves was higher than in control leaves.

The autumn growth of the shoots was very limited both in the control and treated branches, presumably as a consequence of the late fertilization of the leaves with respect to the period of maximum vegetative activity and, above all, of the girdling that has a notoriously depressive effect on the growth of the shoots [28,29].

The absence of differences in leaves' photosynthetic rate can be attributed to the high leaves' N content also in the control. As a matter of fact, the response of leaf photosynthesis to N fertilization is largely dependent on the leaf N content [4].

The number of fruits per inflorescence was not influenced by foliar fertilization in the four years of experimentation. Consequently, it can be deduced that even the fruit drop, which occurred from the time of the first treatment to the harvest, was not modified by foliar fertilization. In other experiments, however, early foliar nitrogenous fertilization has substantially reduced the fruit drop, with a consequent increase in production. This seems to indicate that only in the early stages of fruit development, competition for N can significantly affect the retention of fruits formed by reducing the size of the natural drop [30].

The fresh and dry weight of the fruits and the pulp/pit ratio, in accordance with what reported by [15,30], were not significantly different between control and treated branches. Since the number of fruits per inflorescence and their unit weight did not change with N foliar fertilization, in agreement with what was found by [15], we deduce that the treatment did not change the production by branch either.

It can be assumed that the increase in the size of the fruits detected by other researchers [31,32] after foliar nitrogen fertilization is due to nitrogen deficiency conditions in the olive trees subjected to N fertilization in those experiments, or to the administration of N in times when the fruit demand for this element could be higher, and particularly during the first phases of fruit development when, due to the intense cell division, the basis for the potential subsequent growth of the fruit is created [31,32]. In accordance with [33], the course of the maturation of the olives was not influenced by foliar fertilization. The quality of the oil was not influenced by the foliar treatment and was excellent, even though there was no increase in the content of polyphenols in the oil following N foliar fertilizations as reported by other authors [30,34]. This discrepancy could be justified by hypothesizing that the effect of N on the quality of the oil is not direct, but mediated by that induced on the productive charge and, therefore, on the course of maturation [8,35,36]. Moreover, in some environmental contexts, even the accentuation of the vegetative activity during the summer season, following the nitrogenous foliar fertilization, could contribute to slowing down the ripening of the fruits in the plants subjected to this fertilization as opposed to the control ones.

5. Conclusion

It can be stated, therefore, that the high availability of N during the forwarding phase does not affect the quality of the oil adversely and that, in trees in conditions of good supply of N, it does not induce significant effects on the vegetative-productive activity of the tree. To better define the effect of nitrogenous foliar fertilization on the process of transmission and on the quality of the oil, it would be interesting to carry out treatments on trees in a condition of N deficiency and to investigate the effects obtainable with treatments carried out during the entire vegetative season.

Supplementary Materials: The following are available online at http://www.mdpi.com/2077-0472/9/12/252/s1: Table S1: Experimental site meteorological data.

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Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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