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Effects of nitrogen fertilization and nitrification inhibitor product on vegetative growth, production and oil quality in 'Arbequina' hedgerow and 'Picual' vase-trained orchards

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SUMMARY: Two experiments were carried out in olive orchards in the center of Spain over a three-year period. In this cold and dry area, growers traditionally apply large amounts of N with no experimental knowledge. An 'Arbequina' hedgerow and 'Picual' vase orchards were fertilized with two N-doses applied to the soil in spring with or without the nitrification inhibitor (DMPP). Vegetative growth, fruit and oil characteristics were evaluated. These variables were affected by the N-treatment during the 3rd year. The lowest N-application increased vegetative growth, while when N-leaf composition was higher than 2%, fruit dry weight, oil content and oil phenol content were reduced. 'Picual' did not respond to N-applications. The effect of DMPP on growth or production was not consistent and a lower phenolic content was obtained for 'Arbequina'. Our results demonstrated that in this dry land, N-fertilization is not always necessary and oil quality can be negatively affected with high doses.

KEYWORDS: *Leaf analysis; Oil quality;* Olea europaea *L.; Olive fertilization; Phenolic compounds; Reproductive components*

RESUMEN: Efectos de la fertilización nitrogenada y del inhibidor de la nitrificación sobre el crecimiento vegetativo, la producción y la calidad del aceite en olivares 'Arbequina' en intensivo y 'Picual' en vaso. Durante tres años, se llevaron a cabo dos experimentos en olivares del centro de España. En esta fría y seca zona de cultivo, los agricultores aplican grandes cantidades de nitrógeno sin ningún criterio. Un olivar de 'Arbequina' en intensivo y otro de 'Picual' en vaso se fertilizaron con dos dosis de N aplicado al suelo en primavera con o sin el inhibidor de la nitrificación 3.4 dimethyl phyrazol phosphate (DMPP). El tercer año, el N afectó al crecimiento vegetativo y a las características del fruto y del aceite. La menor dosis de N aplicada, aumentó el crecimiento, pero cuando la composición de N en hoja fue superior al 2%, el peso del fruto, el contenido en aceite y el contenido fenólico se redujeron. 'Picual' no respondió al N. El efecto del DMPP en el crecimiento o la producción no fue consistente y afectó negativamente al contenido fenólico del aceite en 'Arbequina'. Nuestros resultados demuestran que en esta árida zona la fertilización con N en olivo no siempre es necesaria y la aplicación de dosis elevadas de N puede afectar negativamente a la calidad del aceite.

PALABRAS CLAVE: *Análisis foliar; Calidad de aceite; Componentes del rendimiento; Compuestos fenólicos; Fertilización;* Olea europaea *L.*

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1. INTRODUCTION

The olive (*Olea europaea* L.) is one of the most important crops in the Mediterranean regions. Before the 1950s traditional olive orchards in Spain established at low plant densities (< 100 trees per ha) did not receive any fertilizer application. Nutrients were provided by cover crop and pruning. From the 1960s, following the development of Spanish agriculture industrialization, olive orchards increased considerably the number of trees per area and consumption of inorganic fertilizers had an important growth, and suffered over-fertilization in many cases. Since the 1990s olive cultivation has been modernized, increasing the number of trees per area (>1500 tree·ha^{-1}) and introducing irrigation and fertilization practices to improve yield and profitability. In Spain fertilization has been based on traditional management by applying the same fertilization program every year, without considering nutritional soil reserves or plant requirements (Fernández-Escobar, 2010). The perception that annual applications of significant amounts of N-fertilizer represent cheap insurance against the economic risks associated with insufficient N availability is common among growers. This caused many olive orchards to be fertilized in excess, applying N annually at rates that range from 9 to 350 kg·ha^{-1} (Fernández-Escobar *et al.*, 1994). Therefore, an excess of N-fertilization could cause nitrate pollution and increase frost and disease sensibility. Moreover, it could negatively affect oil quality (Fernández-Escobar *et al*., 2006) because of a decrease in polyphenols, which are the main antioxidants (Erel *et al.*,2013).

Fernández-Escobar *et al.* (2009) fertilized the soil and foliar parts with N, applying different doses of N when the previous season's N-leaf concentration was below the threshold limit for deficiency. After 13 years of experiments they observed that annual fertilization with N was unnecessary to maintain high productivity and growth even when olives were not fertilized or when N-leaf composition was below the established deficiency threshold. Fernández-Escobar *et al.* (2012) suggest that N-sources such as rainwater, irrigation and net mineralization of N could compensate N-losses by leaching, pruning, fruit crop and ammonia volatilization.

In order to increase fertilization efficiency different products have been assayed in other crops to synchronize the release of N from fertilizers with crop need. These products are N-stabilizers which inhibit nitrification or urease activity, thereby slowing the conversion of the ammonium N-fertilizer to nitrate (Havlin *et al.*, 2005). N-losses due to leaching are reduced because of retardation of the biological oxidation of ammonium (Zerulla *et al.*, 2001). The compound 3,4-dimethylpyrazole phosphate (DMPP) has already been identified by several authors as one of the most efficient nitrification inhibitors (Hatch *et al.*, 2005). This product is very efficient in inhibiting the nitrification process in soil (Serna *et al.*, 2000), is effective at low concentrations and no toxicological or eco-toxicological side-effects have been reported (Zerulla *et al.*, 2001). The only work we know which focused on the performance of DMPP in olive orchards was conducted by Casar *et al.* (2009). They obtained more growth of the lateral branches and more buds in olive trees using ammonium fertilizers plus DMPP compared to conventional ammonium fertilization. In *Citrus* trees, Rodríguez *et al.* (2011) proved that the use of DMPP increased N-fertilizer uptake and fruit yields when compared with ammonium fertilizers. In these studies, N-loss through leaching was reduced as a consequence of the diminished nitrification rate.

The central area of Spain is the second most important olive area with more than 400,000 ha (MAPAMA, 2016). It is characterized by a cold autumn and winter, low annual rainfall and a warm and dry summer, and irrigation water is scarce. In these conditions there are no studies of N-fertilization practices in olive orchards in this area. Therefore, the aim of this study was to evaluate the effects of different N-applications on the vegetative growth, olive production and oil quality of two different olive orchards: 'Arbequina' hedgerow-trained and 'Picual' vase-trained. N was applied as ammonium sulphate alone or with the nitrification inhibitor DMPP to evaluate whether the delay of the nitrification process had any effect on olive development.

2. MATERIAL AND METHODS

2.1. The site and the experimental orchards

A 3-year experiment was established in two commercial olive orchards located in Valdepeñas (Ciudad Real), central Spain (38º50' N, 3º19' W). The distance between both orchards was about 300 m and the soil type was similar (Typic Rhodoxeralf). In the cv. Arbequina orchard, 11-year-old olive trees were trained in hedgerow at a spacing of 4 x 1.5 m (1667 olives/ha). The soil was sandy loam at the first 0.3 m and clay below this depth. The pH was 7, $CaCO_3$ was < 0.5%, and organic matter was 1.3% in the first 0.15 m of the soil and < 0.6% below this depth. P and K contents were 118 mg·kg^{-1}and 484 mg·kg^{-1} at the first 0.3 m, and < 1.4 mg·kg^{-1} and 247 mg·kg^{-1} below this depth, respectively. In the cv. Picual orchard, 12-year-old olive trees were vase-trained at a spacing of 8 x 6 m (208 olives/ha). The soil was sandy loam at the first 0.18 m and clay below this depth. The pH was 7.1, $CaCO_3$ was < 0.5% at the first 0.48 m and 1.3% below, and organic matter was 1.0% at the first 0.18 m of the soil and < 0.6% below. Available P was 38 mg·kg^{-1} the first 0.18 m and < 1.5 mg·kg^{-1} below, whereas exchangeable

K was 760.5 mg·kg^{-1} in all profiles. Olive trees were pruned annually following their trained systems. Weeds were controlled using pre- and post-emergence herbicides. The climate in the area is typically Mediterranean, with mild rainy springs and hot, dry summers. During the experiment, a weather station 15 km from the site registered temperature, rainfall, wind speed and direction, humidity, and global radiation and calculated ETo by the Penman-Monteith method (Allen *et al.* 1998) from these climatic variables. The mean annual temperature, ETo and rainfall (11-year average) were 13.8 ºC, 1328 mm and 367 mm, respectively.

Both orchards were deficit irrigated by a subsoil drip system of 3.5 L·h^{-1} every 1.5 m in 'Arbequina' and 2 m in 'Picual'. Irrigation doses were applied by the grower according to maximum doses allowed by Guadiana River Basin Authorities each year. So, 'Arbequina' orchard olive trees received 78, 84 and 121 mm in the first, second and third year, respectively, and 'Picual' orchard olives received 15, 21, and 29 mm, respectively.

2.2. Fertilization treatments

During the three years of the experiment, four N-fertilization treatments were applied in the 'Arbequina' orchard (N, N+i, 2N and 2 N+i) and two N-treatments were applied in the 'Picual' orchard (N, N+i). Nitrogen was applied as ammonium sulphate (21% NH_4^+) alone or with 0.8% nitrification inhibitor DMPP (3.4-dimethylpyrazolphosphate). In all cases nitrogen was applied in spring to the soil under the canopy projection, 50% of the dose was applied in April and the other 50% in May. In addition, all trees received a foliar fertilization applied by the grower with N, P and K annual rates of 0.4, 0.4 and 1.7 kg N·ha^{-1}in 'Arbequina' and 0.4, 0.4 and 0.5 kg N·ha^{-1}in 'Picual'; 0.1, 0.1 and 0.9 kg P_2O_5·ha^{-1}; and 0.1, 3.1 and 1.9 kg K_2O·ha^{-1} on both orchards, respectively. Total doses of N annually applied including the grower fertilization are summarized in Table 1. In the first year of the experiment, the doses applied corresponded to doses usually applied by growers. Because no tree response to fertilization was obtained, during the second and third years N-fertilization was reduced following regional authorities' advice in order to reduce nitrate pollution of the groundwater in this area.

N-fertilization treatments and non-treated control trees (CON) were randomized in a complete four-block design. The experimental plot consisted of three trees surrounded by 2 guard trees.

2.3. Leaf analysis

Leaf nutrient concentration was determined each year from 100 mature leaves sampled in July from the middle of the basal portion of non-bearing, current season shoots around the trees and at about 1.5 m above ground. Leaves were collected from each plot in three blocks per treatment and the control. Once in the laboratory, the leaves were rinsed in de-ionized water, dried at 60 ºC, and mineralized at 450 ºC. After that, the ashes were digested with acid and the extract was measured by a ICP-OES spectrometer (Perkin Elmer, Massachusetts, USA) to determine P, K and Ca, Mg, Na, Fe, Mn, Cu, Zn, Mo, Sulfates and B. Nitrogen was determined with a LECO TruSpec®NNitrogen Protein Analyzer System (LECO, Michigan, USA. The methodology was described by Fernández-Escobar *et al.* (2009).

Table 1. Amounts of Nitrogen applied by fertilization (kg·ha^{-1}) for each year and treatment in 'Arbequina' and 'Picual' orchards in control trees (CON) and N-fertilization treatments with ammonium sulphate without (N and 2N) and with the nitrification inhibitor (N+i and 2N+i)

	Nitrogen applied (kg·ha^{-1})		
Treatments	**2008**	**2009**	**2010**
'Arbequina'			
CON	0.4	0.4	1.7
N	151.0	60.6	61.9
N+i	151.0	60.6	61.9
2N	368.0	120.8	122.1
2N+i	368.0	120.8	122.1
'Picual'			
CON	0.4	0.4	0.5
N	138.0	60.3	60.9
N+i	138.0	60.3	60.9

2.4. Vegetative growth and reproductive components

In March the trunk perimeter of 3 control trees per treatment and block were measured at 35 cm above the soil surface. In each control tree, 3 shoots were tagged on the S-side. Vegetative growth was evaluated by measuring shoot length and number of node increments from budburst (March) to harvest (end of October in 'Arbequina' and end of November in 'Picual'). In these tagged shoots reproductive components were measured. The number of inflorescence and the number of fruits per shoot were counted in May and at harvest, respectively. These observations were used to calculate the percentage of buds that developed inflorescence (buds initiated) and percentage of inflorescence at the onset of fruit, at least one flower (fertile inflorescence).

2.5. Production

Two trees per treatment and block were harvested individually ('Arbequina' early to mid-November and 'Picual' at the end of November) and fruit production was weighed each year. From harvested fruits, six

subsamples in 'Arbequina' and 'Picual', close to 25 g, were weighed fresh and again after drying at 105 ºC. In these subsample fruits were counted to determine fruit weight. A maturity index was determined based on the color of skin and pulp (Ferreira, 1979). Oil content was determined by nuclear magnetic resonance (MiniSpec MQ-10, Bruker, Madison, USA) using the method described by Del Rio & Romero (1999). Oil content was calculated on fresh and dry bases. Fruit water content was calculated as a difference between fruit fresh and dry weights.

Another subsample of 2.5 kg was used for oil extraction with the Abencor system (Commercial Abengoa S.A., Seville, Spain) in 2009 and 2010. This unit, consisting of three basic elements, a hammer mill, a thermo-beater, and a pulp centrifuge, simulates the industrial process of virgin olive oil production on a laboratory scale (Martínez *et al.*, 1975). Samples were crushed in the hammer mill at 3000 rpm. The resulting olive paste was placed in stainless steel 1-L containers and malaxated for 45 min in the thermo-beater at 26 ºC, using four stainless steel cross blades at 54.5 rpm (radius 53 mm). After that, the paste was centrifuged in a pulp centrifuge for 1 min at 3,500 rpm (radius 100 mm) to separate the liquid phase (oil and waste water) from the solid waste. Then, the oil was decanted into graduated tubes until complete separation of the water and oil phases was obtained. Oil volume was determined after decantation and the extractability index was calculated as the percentage of olive oil extracted from the total oil content of the fruit (on a fresh matter basis) considering 0.916 $kg \cdot L^{-1}$, the olive oil density at ambient temperature. After measurement, the oil was filtered through filter paper and stored in a N_2 atmosphere at -20 ºC until analysis.

2.6. Oil analysis

Free acidity, peroxide index value, and coefficients of specific extinction at 232 and 270 nm (K_{232} and K_{270}) were evaluated in the last two years according to the Regulation EEC/2568/91. An automated Methrom Rancimat 679 apparatus (Methrom Co., Basel, Switzerland) was used to determine the oxidative stability with a 2.5 g oil sample warmed to 98 ºC and an air flow of 10 $L \cdot h^{-1}$. Results were expressed as induction time in hours (Gutiérrez, 1989). The composition of fatty acids was determined by gas chromatography in a Perkin-Elmer Autosystem (CT, USA). The fatty acids (carbon number:unsaturations) analyzed were myristic (14:0), palmitic (16:0), palmitoleic (16:1), margaric (17:0), margaroleic (17:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0), gadoleic (20:1) and behenic (22:0). From the fatty acid composition, different variables were determined: saturated fatty acids (SAFA), including fatty acids without unsaturations; unsaturated fatty acids (UNFA), including fatty acids with one, two or three unsaturations; monounsaturated fatty acids (MUFA), including fatty acids with only one unsaturation; and polyunsaturated fatty acids (PUFA), including fatty acids with two or three unsaturations. The phenolic fraction of the oil samples was isolated by solid-phase extraction and analyzed by reversed-phase HPLC using a diode array UV detector (Mateos *et al.* 2001). The quantification of phenolic compounds (except ferulic acid) was carried out at 280 nm using *p*-hydroxyphenylacetic acid as an internal standard, whereas that of flavones and ferulic acid was made at 335nm using *o*-coumaric acid as an internal standard. The results were expressed in ppm.

2.7. Statistical analyses

Analyses of variance were performed on the data using MSTAT-C (University of Michigan, USA) to compare the effect of treatments. Least significant differences ($P \leq 0.05$) tests (protected LSD) were used to separate the means of all parameters evaluated between treatments. The error term for LSD was calculated from the residual of the ANOVA. All percentage values were transformed using the arcs of the square root before analysis.

3. RESULTS

3.1. Climatic conditions of the experimental years

Weather data of the experimental periods are represented in Figure1. Annual rainfall in 2008, 2009 and 2010 was 419, 392 and 652 mm, respectively, and annual ETo was 1250, 1305 and 1198 mm, respectively. Annual rainfall distribution was variable particularly from January to May when 236, 100 and 317 mm fell in the 1st, 2nd and 3rd years, which meant 56, 26 and 49% of the total annual rainfall, respectively. In this period, ETo was 393, 433 and 395 mm, respectively. June and July were very dry with 5, 8 and 7% of total annual rainfall, and ETo was 397, 409 and 375 mm, respectively in 2008, 2009 and 2010. From August to October rainfall was 114, 101 and 89 mm, which meant 27, 26 and 13% of the total annual rainfall respectively, and ETo was 398, 379 and 365 mm, respectively. The maximum temperature in summer was measured in July at 39 ºC, 39.6 ºC and 39.7 ºC, respectively. November and December were the coldest months with absolute minimum temperatures of -5.6 ºC, -12.1 ºC and -8.4 ºC, respectively.

3.2. Leaf analysis

In the 'Arbequina' orchard foliar N-concentration in CON varied between 1.5% in 2009 and 1.9% in 2010 (Table 2). In the last year N-fertilization

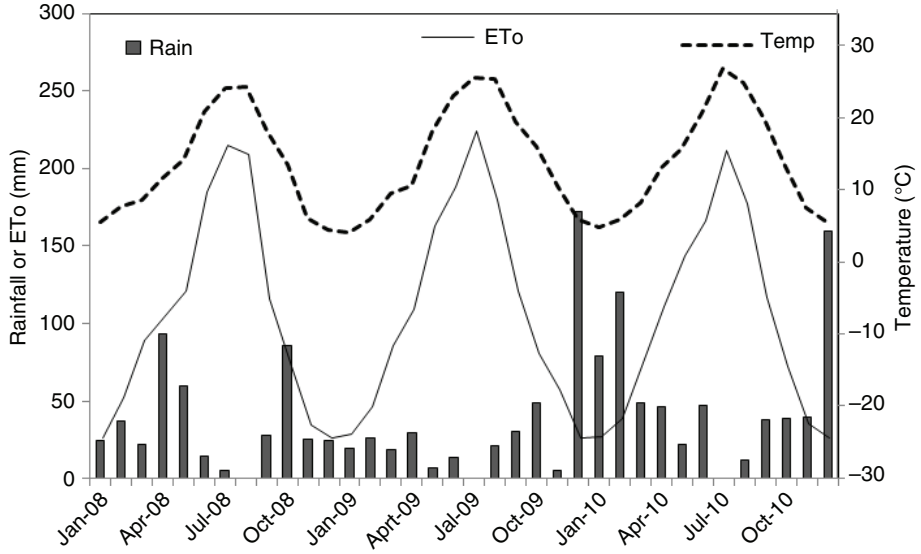

FIGURE 1. Monthly rainfall (Rain, mm), reference evapo-transpiration (ETo, mm), and average temperature (Temp, ºC) from January 2008 to December 2010 at the experimental orchards located in Valdepeñas (Ciudad Real, Spain).

TABLE 2. Leaf N, P and K concentration of 'Arbequina' and 'Picual' olive trees for each year in control trees (CON) and N-fertilization treatments with ammonium sulphate without (N and 2N) and with the nitrification inhibitor (N+i and 2N+i)

	'Arbequina'				'Picual'			
Year	**Treat**	**N (%)**	**P (%)**	**K (%)**	**Treat**	**N (%)**	**P (%)**	**K (%)**
2008	CON	1.57	0.16	1.22	C	1.80	0.14	0.92
	N	1.82	0.15	1.15	N	1.85	0.15	0.96
	N+i	1.91	0.15	1.11	N+i	1.84	0.15	0.95
	II N	1.80	0.18	1.23				
	II N+i	1.68	0.17	1.26				
2009	CON	1.46	0.12	0.93	C	1.67	0.09	0.73
	N	1.58	0.10	0.93	N	1.64	0.08	0.68
	N+i	1.56	0.10	0.95	N+i	1.70	0.09	0.72
	II N	1.48	0.10	0.87				
	II N+i	1.49	0.11	0.98				
2010	CON	1.93 b	0.20 a	1.39 a	C	2.04	0.15	0.95
	N	2.12 a	0.15 c	1.28 c	N	2.06	0.14	0.94
	N+i	2.17 a	0.17 bc	1.30 bc	N+i	2.20	0.14	0.92
	II N	2.10 a	0.19 ab	1.35 ab				
	II N+i	2.07 a	0.18 ab	1.39 a				
2008-2010	CON	1.65	0.16 a	1.18	C	1.84	0.13	0.87
	N	1.84	0.13 c	1.12	N	1.85	0.12	0.86
	N+i	1.88	0.14 bc	1.12	N+i	1.91	0.13	0.86
	II N	1.79	0.16 a	1.15				
	II N+i	1.75	0.16 ab	1.21				
Year	Sign	0.0007	0.0001	0.0011		<0.0001	<0.0001	0.0017

Different letters indicate significant differences ($P \leq 0.05$) within dates according to the LSD test. Absence of letters in a year means no significant effect ($P \leq 0.05$) due to treatments according to ANOVA.

treatments increased foliar nitrogen concentration more significantly than CON and presented a N-leaf composition higher than 2%. In 2N and 2N+i, P- and K-leaf levels were significantly lower than the rest of the treatments and CON. A reduction in the P-leaf level in the most N-fertilized treatments was also observed on the average of the three years of experiment.

In the 'Picual' orchard, N-leaf levels in CON varied between 1.7% in the 2nd year and 2% in 2010 (Table 2). In this orchard N-fertilization treatments did not significantly modify the foliar N-P-K concentration.

3.3. Vegetative growth and reproductive components

In the 'Arbequina' orchard, N and N+i fertilization treatments increased vegetative growth significantly with respect to CON in the last two years (Table 3). These treatments increased trunk perimeter significantly in 2009 and shoot elongation and number of nodes developed in 2010. In this last year, 2N significantly increased shoot elongation with respect to CON by 51%. In 2008, N and N+i increased the percentage of buds initiated. Considering the whole period, the nitrification inhibitor applied in 2N+i significantly developed more nodes per shoot than CON by 39%, and in N+i the percentage of buds initiated was significantly increased regarding the other treatments. Fertile inflorescence was not significantly affected by the fertilization treatments. All parameters were significantly affected by the year. Vegetative growth and the percentage of bud burst and fertile inflorescence were higher in 2010. However, in 2009 the percentage of buds initiated was higher.

In the 'Picual' orchard, N fertilization did not have any significant effect on growth variables or reproductive components (Table 4). Year had a significant effect on reproductive components and vegetative growth, except for trunk perimeter. Shoot elongation and number of nodes were higher in 2008. Nevertheless, the percentages of bud burst and buds initiated were lower.

TABLE 3. Vegetative growth and reproductive components of 'Arbequina' olive trees N-fertilized with (N+i and 2N+i) and without nitrification inhibitor (N and 2N) in 2008, 2009 and 2010

		Vegetative growth				Reproductive components	
Year	Treatment	Trunk perimeter increment (cm)	Shoot elongation (cm)	Number of nodes	Bud burst (%)	Buds initiated (%)	Fertile inflorescence (%)
2008	CON	2.2	16.8	13.8 ab	37.7	68.6 b	60.9
	N	2.4	14.8	10.6 b	35.8	89.2 a	62.2
	N+i	2.1	14.4	9.0 b	41.1	96.2 a	53.5
	2N	1.8	19.3	13.7 ab	44.3	72.7 b	61.9
	2N+i	2.1	24.5	18.7 a	44.1	66.8 b	63.9
2009	CON	1.0 b	7.2	4.5	46.7	100.0	45.9
	N	1.7 a	8.6	5.0	62.3	99.3	47.3
	N+i	1.8 a	6.4	4.0	55.4	100.0	46.0
	2N	0.9 b	7.2	4.4	53.4	99.7	50.3
	2N+i	0.7 b	9.2	5.3	45.5	99.5	60.3
2010	CON	2.8	10.8 c	7.3 b	74.3	99.4	58.2
	N	2.6	27.9 a	14.7 a	76.1	92.2	62.5
	N+i	3.0	25.3 a	12.8 a	76.5	97.9	63.9
	2N	3.3	16.4 b	9.9 b	81.1	97.8	60.2
	2N+i	3.9	14.0bc	8.1 b	76.7	97.3	66.1
2008-2010	CON	2.0	11.6	8.5 b	52.9	89.3 b	55.0
	N	2.2	15.4	8.8 b	58.1	93.6 b	57.4
	N+i	2.3	13.6	7.5 b	57.6	98.0 a	54.5
	2N	2.0	14.3	9.4 ab	59.6	90.1 b	57.5
	2N+i	2.2	18.2	11.8 a	55.4	87.9 b	63.5
Year	Sign.	<0.001	0.005	0.0003	<0.0001	<0.0001	0.0463

Values are reported as means based on 12 olive trees per treatment. Different letters indicate significant differences ($P \leq 0.05$) within dates according to the LSD test. Absence of letters in a year means no significant effect ($P \leq 0.05$) due to treatments according to ANOVA.

TABLE 4. Vegetative growth and reproductive components in 'Picual' olive trees N-fertilized with (N+i) and without nitrification inhibitor (N) in 2008, 2009 and 2010

		Vegetative parameters				Reproductive components	
Year	Treatment	Trunk perimeter increment (cm)	Shoot elongation (cm)	Number of nodes	Bud burst (%)	Buds initiated (%)	Fertile inflorescence (%)
2008	CON	3.8	8.3	7.9	48.1	81.9	51.1
	N	4.3	12.0	12.1	38.6	62.9	61.0
	N+i	3.5	12.0	11.9	30.2	45.5	34.0
2009	CON	2.2	6.2	4.9	44.5	100.0	26.6
	N	2.4	8.5	5.8	44.7	98.8	21.6
	N+i	2.4	7.3	5.4	47.4	97.0	22.0
2010	CON	3.4	6.8	4.4	80.8	93.0	43.2
	N	3.6	9.5	6.3	81.7	95.5	36.7
	N+i	4.0	10.1	6.4	80.0	91.1	38.0
2008-2010	CON	3.2	7.1	5.7	57.8	91.6	40.3
	N	3.4	10.0	8.1	55.0	85.7	39.8
	N+i	3.3	9.8	7.9	52.6	77.9	31.3
Year	Sign.	0.054	0.0116	<0.0001	0.0003	<0.0001	0.0064

Values are reported as means based on 12 olive trees per treatment. Different letters indicate significant differences ($P \leq 0.05$) within dates according to the LSD test. Absence of letters in a year means no significant effect ($P \leq 0.05$) due to treatments according to ANOVA.

3.4. Fruit characteristics and production

In the 'Arbequina' orchard fruits were harvested with a skin color between deep green and yellow-green (average maturity index MI=0.5) (Table 5). Production varied significantly with year. In 2010, olive and oil production were higher.

In 'Arbequina' fertilization treatments only produced significant differences the last year in fruit characteristics (dry weight and oil per fruit) but not in olive or oil production (Table 5). Year significantly determined the effect of N-treatments although fruits were harvested at the same maturity index. The interaction between year and fertilization was not significant in these evaluated variables. The treatments that received the higher N-doses (2N and 2N+i) presented fruits with 14% lower dry weights than CON. Furthermore, fruits from 2N had 24% lower oil content. In the whole period, N+i, 2N and 2N+i had 20% more fruits per tree than CON; however, there were no significant differences in oil production.

In the 'Picual' orchard, olives were harvested with a skin color between pink or purple in more than half of the fruit and black skin with white pulp (MI between 3.1 and 4) (Table 6). In 2010 production was higher than the rest of the seasons. In the first year, N+i increased fruit dry weight, water content and oil per fruit by 52%, 3.3% and 73% regarding CON, respectively, and by 30%, 3.3% and 43% regarding N, respectively. There were no significant differences between treatments for other seasons or variables.

3.5. Oil quality

Oil quality was evaluated in the two latest years by measuring acidity, peroxide index, K_{232} and K_{270} values, and fatty acid and phenolic compositions. In both orchards and treatments, fatty acids such as linoleic, myristic, margaric, margaroleic, araquic, gadoleic and behenic presented very low concentrations (< 0.8%) and were not affected by treatments or year (data not shown). Only the phenols with high concentrations or those showing significant differences between treatments have been studied.

In the 'Arbequina' orchard, year had a significant effect on oil quality parameters. In the oil obtained from CON fruits free acidity reached very low values (0.1-0.2% oleic acid), peroxide value was lower than 6 $meqO_2 \cdot kg^{-1}$, K_{232} and K_{270} were lower than 1.80 and 0.20, respectively and oxidative stability was lower than 36 hours (data not shown). Year also showed a significant effect on fatty acid and phenolic compositions (Table7). During the last year oleic acid concentration, C18:1/C18:2, MUFA/PUFA and UNFA/SAFA were higher than the previous one. However, the phenolic content was higher in 2009 than in 2010. In CON, total phenols, total *O*-diphenols and total secoiridoids in 2010 were respectively 74, 77 and 80% lower than those obtained in 2009. In all seasons, there was a positive linear relationship between stability and total phenols ($y = 42.4x - 679.8$, $R^2 = 0.98$, n=15), total *O*-diphenols ($y = 28.3x - 482.4$, $R^2 = 0.98$, n=15) and total secoiridoids ($y = 41.9x - 721.7$, $R^2 = 0.98$, n=15).

Table 5. Fruit characteristics and production of 'Arbequina' olive trees N-fertilized with (N+i and 2N+i) and without nitrification inhibitor (N and 2N) in 2008, 2009 and 2010

Year	Treatment	Dry weight (g/fruit)	Oil content (% dry matter)	Water content (%)	Maturity index	Oil per fruit (g)	Olive yield (kg/ha)	Oil production (kg/ha)	Olive fruits (number/tree)
2008	CON	0.26	38.0	55.8	0.4	0.10	6758	1140	7305
	N	0.27	38.5	57.5	0.6	0.12	8822	1489	8355
	N+i	0.24	37.6	56.3	0.3	0.12	9199	1520	10047
	2N	0.25	40.7	56.1	0.3	0.10	8747	1570	9258
	2N+i	0.24	38.1	55.8	0.3	0.09	8140	1386	9107
2009	CON	0.35	33.5	54.4	0.6	0.12	5246	782	4112
	N	0.34	32.3	55.4	0.4	0.15	6576	971	5058
	N+i	0.35	34.1	56.2	0.6	0.14	5934	887	4426
	2N	0.35	35.6	55.5	0.6	0.13	5323	807	4071
	2N+i	0.33	33.5	55.1	0.4	0.12	6192	930	4926
2010	CON	0.43 a	38.7	54.4	0.6	0.17 a	11304	2098	7706
	N	0.40 ab	37.6	55.4	0.6	0.15 ab	12106	2113	8533
	N+i	0.41 ab	38.2	56.2	0.4	0.16 ab	12532	2252	8737
	2N	0.36 b	36.0	55.5	0.5	0.13 b	12358	2072	9893
	2N+i	0.38 b	37.0	55.1	0.4	0.14 ab	11167	1949	8439
2008–2010	CON	0.35	36.7	55.4	0.5	0.13	7769	1340	6375 b
	N	0.34	36.1	56.4	0.6	0.12	9168	1525	7315 ab
	N+i	0.33	36.7	56.4	0.4	0.12	9222	1553	7737 a
	2N	0.32	37.4	56.3	0.5	0.12	8809	1483	7741 a
	2N+i	0.32	36.2	55.7	0.3	0.12	8500	1421	7490 a
Year	Sign.	0.0001	0.0058	0.0226	ns	0.0025	<0.0001	<0.0001	<0.0001

Values are reported as means based on 12 olive trees per treatment. Different letters indicate significant differences ($P \leq 0.05$) within dates according to the LSD test. Absence of letters in a year means no significant effect ($P \leq 0.05$) due to treatments according to ANOVA.

Table 6. Fruit characteristics and production of 'Picual' olive trees N-fertilized with (N+i) and without nitrification inhibitor (N) in 2008, 2009 and 2010

Year	Treatment	Dry weight (g/fruit)	Oil content (% dry matter)	Water content (%)	Maturity index	Oil per fruit (g)	Olive yield (kg/ha)	Oil production (kg/ha)	Olive fruits (number/tree)
2008	CON	0.71 b	38.7	43.1 b	3.1	0.28 b	3733	818	14858
	N	0.83 b	39.7	43.1 b	3.2	0.34 b	3519	793	11998
	N+i	1.08 a	43.4	46.4 a	3.2	0.48 a	3583	830	10004
2009	CON	1.02	39.5	40.4	4.0	0.41	3314	778	9311
	N	0.99	39.5	40.4	4.0	0.39	3466	822	9981
	N+i	0.96	39.2	41.6	3.9	0.38	3861	875	11300
2010	CON	1.33	41.6	51.5	3.4	0.55	6010	1216	11000
	N	1.21	40.4	49.2	3.4	0.49	6392	1299	13190
	N+i	1.16	40.3	49.5	3.4	0.47	6017	1219	13081
2008–2010	CON	1.02	39.9	45.0	3.5	0.41	4352	937	11723
	N	1.01	39.9	44.2	3.5	0.40	4459	971	11723
	N+i	1.07	41.0	45.8	3.5	0.44	4487	975	11461
Year	Sign.	0.0003	ns	<0.0001	0.0001	0.0044	<0.0001	0.0003	0.046

Values are reported as means based on 12 olive trees per treatment. Different letters indicate significant differences ($P \leq 0.05$) within dates according to the LSD test. Absence of letters in a year means no significant effect ($P \leq 0.05$) due to treatments according to ANOVA.

TABLE 7. Fatty acid composition (%) and total phenolic compounds (ppm) of olive oil from 'Arbequina' olive trees N-fertilized with (N+i and 2N+i) and without (N and 2N) nitrification inhibitor in 2009 and 2010

Year and treatment	C18:1/C18:2	SAFA (%)	UNFA (%)	MUFA/ PUFA	UNFA/ SAFA	Total phenols (ppm)	Total ortodiphenols (ppm)	Total Secoiridoids (ppm)
2009								
CON	6.9	18.1	81.8	6.6	4.5	835.2	537.6	784.6
N	6.8	18.2	81.8	6.5	4.5	523.9	308.5	468.4
N+i	6.8	18.1	81.9	6.5	4.5	663.6	422.8	603.9
2N	6.6	18.0	81.9	6.4	4.6	690.1	421.9	631.4
2N+i	6.8	18.0	81.9	6.5	4.5	707.0	444.7	642.3
2010								
CON	9.5	15.6	84.4	9.1	5.4	220.3 a	125.2 a	157.9 a
N	9.1	15.4	84.6	8.8	5.5	165.6 ab	93.3 b	127.4 ab
N+i	9.1	15.3	84.6	8.8	5.5	121.9 b	50.0 d	56.0 c
2N	10.6	14.9	85.1	10.0	5.7	174.2 a	84.6bc	129.0 ab
2N+i	9.7	15.1	84.8	9.3	5.6	135.1 b	62.9 cd	96.4bc
2009-2010								
CON	8.2	16.8	83.1	7.8	5.0	527.7	331.4	471.3
N	8.0	16.8	83.2	7.6	5.0	344.7	200.9	297.9
N+i	8.0	16.7	83.2	7.6	5.0	392.8	236.4	330.0
2N	8.6	16.4	83.5	8.2	5.1	432.1	253.3	380.2
2N+i	8.3	16.6	83.4	7.9	5.1	421.1	253.8	369.3
Year (*Sign.*)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Values are reported as means based on 4 olive oils per treatment. Different letters indicate significant differences ($P \leq 0.05$) within dates according to the LSD test. Absence of letters in a year means no significant effect ($P \leq 0.05$) due to treatments according to ANOVA. MUFA: Monounsaturated fatty acids (%), PUFA: Polyunsaturated fatty acids (%), SAFA: Saturated fatty acids (%), UNFA: Unsaturated fatty acids (%)

In this orchard, N-fertilization did not produce significant differences in oil quality variables or fatty acid composition individually considered or when ratios were analyzed. However, N-fertilization with and without nitrification inhibitor negatively affected the oil's phenolic composition (Table 7). During the last year *O*-diphenols were reduced in all N-fertilization treatments and N-treatments with nitrification inhibitor presented lower total phenols, total *O*-diphenols and total secoiridoids than CON.

In the 'Picual' orchard, year had a significant effect on oil quality parameter variables except for oxidative stability, which was an average of 61.5 hours (data not shown). In this orchard, the relationship between stability and phenol content was not significant. Free acidity in the oil from CON reached very low values, lower than 0.3% oleic acid, peroxide value was lower than 4 $meqO_2 \cdot kg^{-1}$, and the values of K_{232} and K_{270} were lower than 1.7 and 0.2, respectively (data not shown). Year also had a significant effect on fatty acid composition and in the last year oleic acid concentration was higher than 2009 (Table 8). Regarding phenolic compounds there were no differences between years.

In this orchard, N-fertilization did not produce significant differences in any variables of oil quality, in phenolic composition or in the fatty acid composition individually considered. However, in the last year N+i increased significantly the UNFA/SAFA ratio regarding CON and N by 2%.

4. DISCUSSION

Nitrogen fertilization is a commonly agronomic practice in olive orchards with the aim of improving fruit yield (Fernández-Escobar *et al.*, 2006). However, positive responses are not always observed because either N-plant levels are adequate or the fertilization method, or the type of fertilizer applied do not improve the N-nutritional status. In addition, climatic and soil conditions have an important influence on plant nutrition. In this sense, soil moisture in the root area is essential to improve soil nutrient uptake because it encourages the emergence and development of new roots, extends their period of maximum activity and helps dissolve and uptake fertilizer compounds (Troncoso *et al.*, 2010). Efficiency of fertilization will also be influenced by root density.

In the experiments reported in this paper the climatic conditions determined the response to N-fertilization both in the super high density

Table 8. Fatty acid composition (%) and total phenolic compounds (ppm) of olive oil from 'Picual' olive trees N-fertilized with (N+i) and without (N) nitrification inhibitor in 2009 and 2010

Year and treatment	C18:1/C18:2	SAFA (%)	UNFA (%)	MUFA/ PUFA	UNFA/ SAFA	Total phenols (ppm)	Total ortodiphenols (ppm)	Total Secoiridoids (ppm)
2009								
C	17.9	14.7	85.3	15.5	5.8	755.8	419.3	743.9
N	17.7	14.6	85.4	15.5	5.9	828.0	464.3	799.7
N+i	17.9	14.5	85.4	15.6	6.0	670.7	360.9	659.9
2010								
C	33.4	13.1	86.9	27.0	6.7 b	986.4	724.7	897.0
N	33.0	13.1	86.8	26.8	6.6 b	845.3	597.8	757.3
N+i	33.5	12.8	87.0	27.3	6.8 a	753.0	524.8	680.9
2009–2010								
C	25.7	13.9	86.1	21.3	6.2	871.2	572.0	820.5
N	25.4	13.8	86.1	21.1	6.3	836.7	531.0	778.5
N+i	25.7	13.7	86.2	21.4	6.3	711.9	442.9	670.4
Year(*Sign.*)	<0.0001	<0.0001	0.0001	<0.0001	0.0001	ns	ns	ns

Values are reported as means based on 4 olive oils per treatment. Different letters indicate significant differences ($P \leq 0.05$) within dates according to the LSD test. Absence of letters in a year means no significant effect ($P \leq 0.05$) due to treatments according to ANOVA. MUFA: Monounsaturated fatty acids (%), PUFA: Polyunsaturated fatty acids (%), SAFA: Saturated fatty acids (%), UNFA: Unsaturated fatty acids (%)

orchard of 'Arbequina' and the lower density of 'Picual'. They were located in a semi-arid zone with high temperature fluctuations (higher than 39 ºC in summer and lower than -10 ºC in winter) and scarcity of rainfall in summer (Fig. 1). In this dry land of the center of Spain water for irrigation is scare and fluctuates greatly from year to year but is much lower than olive water requirements. During our experiment, water volume applied by irrigation was very low considering the high water demand. Irrigation was applied at a rate of 21.6, 16.1 and 33.2% of ETo in 'Arbequina' for 2008, 2009 and 2010, respectively, and 4.2, 4.0 and 8.0% of ETo in 'Picual' for each year, respectively.

Under these deficit irrigation conditions, rain plays a key role in the plant physiology. In 2009 rainfall was lower than the other years (Fig. 1) and negatively affected vegetative growth in both orchards (Tables 3 and 4). The high water shortage during spring and summer of this year also affected the percentage of fertile inflorescence, which was especially low, probably because the olives are very sensitive to water starvation during the phases of bud development and flowering (Orgaz and Fereres, 2010). In both orchards, fruit size was significantly higher the last year than the other ones (Tables 5 and 6). This was probably due to a higher spring rainfall and higher irrigation volume applied during the fruit growth period. Moreover, oil content per fruit, number of fruits and oil production were also higher than the other years (84% and 168% more in 'Arbequina' and 49% and 56% more in 'Picual', respectively). Under these dry conditions year had a significant effect on most of the evaluated variables. It impacted olive vegetative and productive behavior more than the N-fertilization treatments. Fernández-Escobar *et al.* (2009) also obtained different responses to N-fertilization from a 13-year experiment on a summer dry land and non-irrigated olive trees.

The leaf analysis provides information about the nutritional status of the olives. In both orchards during 2008 and 2009 all treatments had N-leaf levels (Table 2) within the optimum N-concentration range for olive trees of 1.5-2% (Fernández-Escobar, 2010). During the experimental period N-leaf concentration from CON oscillated between 1.5 and 1.9% and it did not drop below a deficit N-concentration level fixed at 1.4%. Probably other sources of N such as water irrigation, rainfall and mineralization of the organic matter could contribute to the N-plant necessities as pointed out by Fernández-Escobar *et al.* (2012). However, during the last year in the 'Arbequina' orchard, leaves from N-fertilized treatments had a N-concentration higher than 2%, most likely because of the accumulative effect of fertilization and the higher soil humidity due to higher rainfall and irrigation. Fernández-Escobar *et al.* (2009) observed significant increases in N-leaf composition but levels did not exceed the optimum value. However, the slight increase in N-leaf composition in the most fertilized treatments (2N and 2 N+i) was low considering the high doses applied (Table 1).

The N-treatments slightly affected the vegetative and reproductive variables evaluated in 'Arbequina' but not in 'Picual' orchards (Tables 3 and 4).

Considering the three experimental years in the 'Arbequina' orchard the number of nodes was higher when higher doses were applied (Table 3). Connell *et al.* (2002) obtained longer shoots when urea was applied to the leaves. However, in the last year, when N-leaf content was higher than 2%, shoot growth and number of nodes developed were significantly lower in the higher doses. Fernández-Escobar *et al.* (2004) also observed a significant reduction in shoot growth and some damage in containerized plants that received high doses of N compared to low doses.

In the 'Picual' orchard fertilization with N with or without the nitrification inhibitor did not produce any significant effect on growth or reproductive components (Table 4). The lack of responses could be due to water scarcity and the high soil availability for olive trees (around 12.6 m^2) which could provide enough nutrients for plant development. The lower tree density and lower root density compared to the 'Arbequina' orchard could explain this lower response to N fertilization. Cultivar effect should also be considered. In olive trees, different cultivars exhibited significant variations in nutrient absorption from the soil (Saidana *et al.*, 2009).

The nitrification inhibitor did not produce differences in vegetative growth and production in either of the experimental orchards. In other agricultural and horticultural crops the use of a nitrification inhibitor had positive effects on yield (Pasda *et al.*, 2001). The positive effect of the nitrification inhibitor in the number of fruits per trees and in fruit yield was also observed in *Citrus* trees (Rodríguez *et al.*, 2011). The increase in crop yield applying N with nitrification inhibitor has been related to a higher availability of NH_4^+ and a higher concentration of N in leaves than fertilizing without the inhibitor (Rodríguez *et al.*, 2011). The lack of response to the inhibitor in our experiment could be due to climatic (low water availability) or soil conditions (clay soil with a high capacity of water retention). A greater response to nitrification inhibitors would be expected when N-loss by de-nitrification or leaching is severe as pointed out before by Serna *et al.* (2000). To evaluate the efficacy of the nitrification inhibitor it seems to be essential to consider the soil texture because most works (Serna *et al.* 2000, Pasda *et al.* 2001, Rodríguez *et al.* 2011) have shown better responses to nitrification inhibitor applications in sandy than in clay soils.

In this experiment the higher N-application even with inhibitor did not increase growth or production. Apart from the low profitability associated with this practice the environmental problems associated with N-leach and nitrate pollution risk should be considered.

In both orchards, the values obtained by the extracted oils in the parameters legally established for evaluating the level of commercial quality (free acidity, peroxide value, K_{232} and K_{270}) classify the olive oil as extra virgin olive oil, the best possible level of quality according to Commission Regulation EC No. 1989/2003, 6 November 2003 (data not shown).

Again, N-treatments slightly affected oil quality in 'Arbequina' but not in 'Picual' (Tables 7 and 8). Treatments with N did not produce significant differences in free acidity, peroxide value, K_{232} and K_{270} in 'Arbequina' or 'Picual' olive oil (data not shown). This result is in accordance with Tekaya *et al.* (2013), who demonstrated that nutrient-based fertilization did not influence these quality indices for olive oil.

N-fertilization did not affect the fatty acid composition of 'Arbequina' and 'Picual' olive oil (Tables7 and 8). Meanwhile in other experiments, a reduction in linoleic acid coincided with N-application, and, consequently, a decrease in the relation between UNFA and SAFA was observed (Toplu *et al.*, 2009). Erel *et al.* (2013) reported that the level of desaturation for the three major C18 fatty acids (oleic, linoleic and linolenic acids) increased with fruit N-content. However, these authors pointed out that no solid linkage between N and oleatedesaturase could be found in the literature and the N-level may indirectly affect desaturation via assimilate availability.

Phenols seem to be the chemical parameters which are the most influenced by N-fertilization in 'Arbequina'. In the last year of the experiment when N-leaf composition exceeded the optimum N-concentration range, the phenolic acids were reduced with N-fertilization with and without the inhibitor, so oil quality was negatively affected (Table7). Phenols are recognized as antioxidant compounds and their presence in olive oil has been related to their general properties of improving stability, nutritional value and sensory properties (Servili *et al.*, 2004). Total ortodiphenols decreased in all N-fertilized treatments regardless of doses or products. However, total secoiridoids were only reduced in treatments with inhibitor. The ortodiphenol family can be identified as the main source of the overall antioxidant activity of extra virgin olive oils (Servili *et al.*, 2004). The concentration of phenolic compounds in olive oil is influenced by water availability (Servili *et al.*, 2004) which is higher under dry land. This fact could explain the higher level of phenols in 2009 after a dry season. In the review of Stefanelli *et al.* (2010) the authors discussed the adverse effects of N on overall quality, and especially on secondary plant metabolites, and proposed the optimal N-applications to maximize phenolic composition in various crops. Most of them had the highest flavonoid content with the lowest N application rate. Fernández-Escobar *et al.* (2006) obtained a significant decrease in polyphenol content with increasing N availability in fruit, and Dag *et al.* (2009) observed an important decrease in polyphenol content and oxidative stability by

increasing N-fertilizer doses. Jones and Hartley (1999) pointed out that the synthesis of proteins and phenols have phenylalanine as a common precursor but follow opposite biosynthetic pathways. Under high N conditions, the synthesis of proteins demands a high amount of the precursor, reducing the synthesis of phenols and hence polyphenols. In olives it has been observed by Erel *et al.* (2013) that fruit phenol content is inversely related with N in leaf, and they suggested that either phenol precursors or polyphenols themselves could be produced in leaves and translocated to the fruit, and it is on the leaves where the competition between both pathways occurred. These authors obtained a negative relationship between high nutritional N-doses and oil quality in olive trees, and observed that when N content in leaves increased, oleic acid and polyphenol content were reduced, negatively affecting oil quality.

5. CONCLUSIONS

Our study supports previous studies' results that observed a negative effect of N over-fertilization on production and quality of olives. In the olive area of the center of Spain, N-fertilization is a common practice with no previous experimental knowledge. N-fertilization management should not only consider production, but also its impact on oil quality. In these dry conditions where water is scare for irrigation, rainfall has more impact on growth and production than fertilizer. Only in those rainy spring years did the tree respond to soil fertilization in the super-intensive 'Arbequina' orchard. In dry years orchard N requirements are low and can be satisfied by minimal foliar fertilization, as the control trees of this experiment. When large amounts of N-fertilizer were applied 'Arbequina' oil quality was reduced by decreasing phenol content. Young 'Picual' trees did not require N-fertilizer application apart from foliar spray of the control trees when leaf analyses indicate levels near 1.4%. In our experimental conditions, N-inhibitor is not recommended for the lack of effects. The recommendations of this experiment should be useful for sustainable N-fertilization management, for reducing environmental problems and for obtaining high quality oil.

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