Nitrogen Management for Improving Root and Shoot Components of Young ‘Arbequina’ Olives

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Abstract. The impact of nitrogen application on the growth of olive trees has been well studied. However, little is known about the role of levels and forms of N on the development of roots and physiological traits during establishment of young trees. The objective of this 2-year study was to evaluate the influence of N source and level on shoot morphology (tree height, stem diameter, and branch number) and physiology [leaf area and fresh weight, photosynthesis (P_n), transpiration (E), and stomatal conductance (g_s)], root components (length, diameter, surface area, and fork number) and N content of young olive (*Olea europaea* cv. Arbequina) cuttings. Three-month-old olive cuttings were planted in 15-L pots filled with a growing substrate composed of peatmoss + bark + sand (2:1:1 by volume) and placed in a screen house. Two N levels, 2.8 and 5.6 g/tree, and control (0 N) and four N sources, calcium nitrate (CN, 12%N, 17%Ca²⁺), ammonium nitrate (AN, 35%N), urea (46%N), and the slow-release Osmocote (OSC, 18%N, 6%P, 12%K) were evaluated. Effects of low- (2.8 g/tree) and high N (5.6 g/tree) levels on shoot components (plant height, diameter, branch number, leaf area, and fresh weight) and gas exchange (P_n , g_s , and E) were similar implying that the low rate of N was adequate for the establishment of young olive cuttings. Nitrogen sources, particularly AN and CN had significant effects on shoot and root morphology, physiology, and leaf and root N concentration. In fact, AN and CN were the best fertilizer sources for olive transplants in term of root and shoot growth. Overall, 2.8 g/tree N level and AN or CN sources were the best treatments for newly established olive ‘Arbequina’ trees. Root components treated with high N rate (5.6 g/tree N) using the slow-release fertilizer (OSC) was similar to those treated with the low AN rate (2.8 g/tree N). Therefore, for nursery containerized olive trees of ‘Arbequina’ or other cultivars with comparable growth rates, we recommend to apply the CN or AN source at 2.8 g/tree N or the controlled-released fertilizer OSC at 5.6 g/tree N.

Plant nutritional status, specifically nitrogen (N), plays key roles in olive growth and productivity (Rodrigues et al., 2011a). Plants develop unique physiological and diverse developmental mechanisms that control N root uptake capacity, adapting to fluctuating N resource availability (Nacry et al., 2013). Because of the transient nature of N in the soil/plant system, Rodrigues et al. (2012) recommends that N be applied every year to increase the nutrient-use efficiency of olive

trees. However, the requirement of optimal N concentration in leaves and roots is controversial. For example, in young olive trees ‘Meski’ and ‘Koroneiki’ N deficiency (no N applied) reduced net CO₂ assimilation rate but did not significantly change the maximum quantum efficiency of PSII photochemistry (Boussadia et al., 2015). Although N-deficient olive seedling had higher levels of starch, mannitol, sucrose, and glucose the same cuttings had lower leaf N and chlorophyll a content, leaf dry weight, and photosynthetic capacity (Boussadia et al., 2010). Troyanos and Roukounaki (2011) found that olive cuttings subjected to high levels of N (6.25 g/tree N) had higher leaf dry weight and shorter root system compared with control-untreated cuttings.

The optimal N content is known as the minimum shoot N content required by a crop to reach maximum growth (Boussadia et al., 2015). Developmental root components including root hairs and lateral roots are par-

ticularly highly sensitive to the internal and external concentration of nutrients such as N and phosphorus (P) (López-Bucio et al., 2003). In olive, it has been shown that the leaf N concentration greater than 1.7% (dry weight) has negative effects on flower and oil quality (Molina-Soria and Fernández-Escobar, 2010). Olive cuttings fertilized with 0.75 g N per pot had higher shoot growth than those that received 2 g N (Fernández-Escobar et al., 2004). Interestingly, elongation of fine cuttings roots of cv. Koroneiki was enhanced under severe N deprivation (Boussadia et al., 2010).

In addition to N level, N form can significantly affect root and shoot growth and development (Lu et al., 2009; Takács and Técsi, 1992). Moreover, N form and nitrate (NO₃⁻)/ammonium (NH₄⁺) ratio play a key role in shoot and root development and the ultrastructure of chloroplasts (López-Bucio et al., 2003; Takács and Técsi, 1992). Nitrogen is available for plants in either organic (free amino acids) or inorganic (NO₃⁻ and NH₄⁺) forms (Kiba et al., 2011), with NO₃⁻ being the main source of inorganic N (Andrews, 1986). The reduction rate of NO₃⁻ to ammonia in plant shoot to fulfill its essential functions as a plant nutrient depends on the level of nitrate supply, cultivar, and growth stage (Bouranis et al., 2004). Olive cuttings that received 16 mm urea-N had better growth compared with those that received either NO₃⁻, or NH₄⁺, or the combination of NH₄⁺ + NO₃⁻. However, at lower N levels using (1 or 8 mm), higher plant growth was noticed when they were fertilized with NO₃⁻-N (Tsabarducas et al., 2017).

In agricultural soils, low N retrieval by plants increases the total N losses from the field, triggering undesirable effects on the environment (Fernández-Escobar et al., 2004). A study by Fernández-Escobar et al. (2012) found that, when N was applied annually to fertile olive orchards, N net mineralization was decreased and N net immobilization into soil organic matter increased. Consequently, N leaching increased because of excess of N applied, causing a disruption of the N balance in the soil and environmental damage. Slow-release N fertilizers provide a slow supply of N to plants for a longer period as compared with traditional N fertilizers. As a result, N-use efficiency increased and the total N losses by leaching decreased (Fernández-Escobar et al., 2004). Studies on the effect of N application on olive growth have been common recently (Troyanos and Roukounaki, 2011; Tsabarducas et al., 2017). It has been claimed that N fertilizer is not essential when olive orchard soils are fertile and leaf N content ranges from 1.22% to 1.35% N (% leaf dry matter) (Fernández-Escobar et al., 2012; Molina-Soria and Fernández-Escobar, 2010). However, few have included root components (length, diameter, surface area, and fork number) and leaf gas exchange analysis as an aid to determine the optimal N level for olive growth, specifically at early growth stages. The influence of N source on young olive plants is also not well known, especially,

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those from slow-release N. We hypothesize that low N levels can enhance root components and shoot growth. We also hypothesize that N form (NH_4^+ or NO_3^-) has differential effects on shoot and root growth. The objective of this research was to evaluate the influence of N source and level on shoot morphology and physiology, root component and plant N content of young olive cuttings cv. Arbequina.

Material and Methods

Plant material and experiment setup. A 2-year study was established in pots under a screen house to evaluate the impact of N rate and fertilizer source based on different proportions of N sources NH_4^+ , NO_3^- and mix of NH_4^+ and NO_3^- . The research was carried out during the 2015–16 period at the Texas A&M AgriLife Research and Extension Center, Uvalde, TX (lat. $99^\circ 45' 21.6''$ W, long. $29^\circ 12' 57.6''$ N). Three-month old olive (*O. europaea* cv. Arbequina) cuttings were planted on 13 May 2015 in 15 L pots (diameter 30 cm) filled with growing substrate composed of peatmoss + bark + sand (2:1:1 by volume). The chemical composition of the growing media is described in Table 1. The selected plants were uniform in growth (shoot height, stem diameter, number branches, and leaves). The average plant height for olive cuttings was 70 cm, stem diameter was 0.5 cm, number of leaves was 75, and branches number was seven. Olive cuttings in pots were placed on a bench table in screen house with fabric mesh (50% shade percentage) and left open from the top. Mean temperatures, relative humidity, and light intensity during the study period are given in Fig. 1.

Nitrogen source and rate. During the study period, the following nine treatments were evaluated: control (no N), NO_3^- source applied as CN (12%N, 17% Ca^{+2}) at 2.8 and 5.6 g/tree, $\text{NO}_3^- + \text{NH}_4^+$ applied as AN (35% N) at 2.8 and 5.6 g/tree, NH_4^+ applied as urea (46% N) at 2.8 and 5.6 g/tree, and the controlled-release fertilizer OSC: 18-6-12 applied at 2.8 and 5.6 g/tree. The experiment was designed as a complete randomized block design with four replications containing two plants per replication (eight total per treatment) in the first year and one plant per replication (four total per treatment) in the second year (2016). For each treatment and

year, four young trees were used for destructive measurements (root and leaf analysis) each year. For each year, the total OSC fertilizer was applied once at the beginning of the growing season, whereas the other three sources (CN, AN, and urea) were applied weekly as a drench treatment at a rate of 120 mL per pot over a 20-week period every year. To balance the additional nutrient content (Ca^{+2} , P, and K) provided in the CN (Ca^{+2}) and OSC (P, K) treatments were compensated with equivalent amounts of calcium sulfate, rock phosphate, and potash, which were applied once at the time of planting and the beginning of the second year.

Growth and physiology measurements. In both seasons, leaf gas exchange (P_n , E , and g_s) were measured 15 weeks after the N application in each year. Gas exchange measurements were conducted between 1100 and 1300 HR from two fully expanded leaves. Gas exchange measurements were determined using a portable photosynthesis system (LI-6400XT; LI-COR, Lincoln, NE) and using the procedures of Othman et al. (2014). At the end of each growing season (20 weeks after N application), plant size (height and width) was measured and then four olive cuttings from each treatment were harvested and divided into shoot and roots, and then, the number of branches, stem diameter, leaf fresh weight, leaf area, and root components were determined. The leaf area was

measured using a leaf area meter (LI-3100C Area Meter; LI-COR). The root components (length, surface area, diameter, volume, and fork number) were measured using a WinRHIZO image analysis system (V5.0; Regent Instruments, Quebec, Canada). Leaf and root N% was determined using the procedures of Al-Ajlouni et al. (2017). Leaves and root samples were collected, oven dried for 3 d at 65°C , then, total N was determined using a discrete analyzer (Easy Chem. Plus; Analytical Technologies, Anagni, Italy).

Statistical analysis. A randomized complete block design with four replications and two factors (3 N levels and 4 N sources) was used. For the experiment, four replications represented by two trees each in 2015 and one tree each in 2016 were used. The analysis of variance and the least significant difference test ($P = 0.05$) in SAS (Version 9.4 for Windows; SAS Institute, Inc., Cary, NC) were used to identify differences between N levels, sources, and their interactions.

Results

Morphology and physiology. During the study period (2015–16), plant morphology (plant height, stem diameter, branch number, leaf area, and leaf fresh and dry weight) and gas exchange (P_n , g_s and E) from the 2.8

Fig. 1. Mean minimum and maximum temperatures, relative humidity, and light intensity during the study period from the weather station near the net house (≈ 200 m) at Uvalde, TX.

Table 1. Growing media chemical analysis at the beginning of the experimental study.

Variable	Concn.
pH	5.2
Conductivity ($\mu\text{mho}\cdot\text{cm}^{-1}$)	128.0
Nitrate-N (ppm)	1.0
Phosphorus (ppm)	4.7
Potassium (ppm)	22.7
Calcium (ppm)	816.0
Magnesium (ppm)	74.7
Sulfur (ppm)	7.3
Sodium (ppm)	1.7
Organic matter (%)	8.4

g/tree N rate were similar to those plants from 5.6 g/tree N (Table 2). No significant difference was noticed in g_s and E over the study period across both N levels (2.8 and 5.6 g/tree) and N sources (AN, CN, urea, and OSC). However, in 2015, plants grown with AN fertilizer had higher leaf area and fresh weight than with other N fertilizer sources. In addition, in 2016 CN had higher plant height, stem diameter, leaf area, and leaf fresh and dry weight than OSC and urea (Table 2). In both years, plant morphological components and P_n from untreated plants were significantly lower than those with N-treated plants. OSC had higher P_n rate than urea in 2015, following the same trends, although not significantly in 2016 (Table 2).

Plants fertilized using the high N rate (5.6 g/tree N) had higher leaf and root N% when compared with the low rate N (2.8 g/tree N) and control (0 g/tree N) across the study period (2015–16) (Table 3). The leaf N% was higher than the root N% across the N sources and levels (except for control in 2016). In addition, AN source had higher N% than OSC (slow-release fertilizer) in leaf and root across the study period (Table 3). Interestingly, overall CN gave more NO_3^- in roots and least in leaves, whereas the opposite was found for OSC, with more NO_3^- in leaves and less in roots. However, leaf NO_3^- concentration from the 2.8 g/tree rate was similar to those plants fertilized with 5.6 g/tree N (Table 3) with an average of 0.024% and did not differ significantly from the control.

Root components. Root component analysis of olive transplants revealed significant differences between N fertilizer sources and levels in both years (Table 4; Fig. 2). In addition, there was a significant N source and level interactions for root length and root surface area in 2015 and all root components (except root diameter) in 2016 (Table 4; Fig. 2). The N source and level treatment

interactions revealed that, in 2015, root length, surface area, and root volume (Fig. 2) from the control (0 g/tree N) plants was significantly higher than low (2.8 g/tree N) or high (5.6 g/tree N) rates of AN and OSC. Conversely, in 2016, after 2 years of N application, both N sources, AN and OSC, significantly increased root components as compared with control. After 2 years of N application, the lower rate (2.8 g/tree N) of AN and CN and the high rate (5.6 g/tree N) of AN and OSC had higher root dry weight, root volume, root surface area, total root length, and fork number than other treatments (Fig. 2).

Discussion

Morphology and physiology. In this study, plant morphology and leaf gas exchange parameters (P_n , g_s and E) from the 2.8 and 5.6 g/tree N were statistically similar. It has been shown that morphological traits such as leaf area and leaf dry weight are vulnerable to N availability (Saidana et al., 2009). A hydroponic study for growth analysis using olive seedlings also confirmed the negative effects of nutritional stresses on dry matter, chlorophyll concentration, and higher stomata resistance (Saidana et al., 2009). Consistent with the reports showing reduced yield and leaf N content in the orchards receiving no nitrogen (Rodrigues et al., 2011b), in this study the plant morphological components and P_n from untreated control plants were significantly lower than N-treated plants.

From the substrate data reported in Table 1, there was 8.4% of organic matter that can release nutrients over time. In fact, NO_3^- content for the growing substrate was $1 \text{ mg} \cdot \text{kg}^{-1}$ at the beginning of the experiment. However, the untreated–N control treatment had lower shoot and root growth compared with N-treated trees. Therefore, we believe

that the released nutrients (specifically N) from the growing substrate had slight effects on the olive trees growth.

The increase in leaf and root N% in response to the higher application rate (5.6 g/tree N) compared with the control (0 g/tree N) or lower rate N (2.8 g/tree N) in both years, 2015 and 2016, agrees with the findings by Connell et al. (2002) on ‘Mission’ table olive. Except for the control in 2016, olive leaves had higher N% than roots across the N sources and levels. Fernández-Escobar et al. (2004) concluded that regardless of N formulation in conventional or slow-release N fertilizers, N is accumulated mainly in leaves of the olive transplants. In both growing seasons, the controlled-release fertilizer (OSC) had consistently lower N% than the other three N sources (Table 3). The slow release of N might be beneficial for young seedlings to avoid potential toxic effect to the root system (Fernández-Escobar et al., 2004) as well as runoff and leaching losses.

A long-term study in Spain by Fernández-Escobar et al. (2012) in fertile olive orchard soil (clay loamy to sandy clay soil) revealed that annual N application is not essential to produce a good crop. Molina-Soria and Fernández-Escobar (2010) suggested that the leaf N deficiency threshold for mature olives trees range from 1.22% to 1.35% N (% leaf dry matter), whereas the toxicity N threshold level is greater than 1.7% N. In our study, leaf N% was never below 1.2% for all N sources (AN, CN, and OSC) and levels (2.8 and 5.6 g/tree N), except for the lower level of OSC (1.16%) in 2016 (Table 3). High leaf N concentration specifically, during the first year of 2015, could explain the nonsignificant effect of the higher N level (5.6 g/tree N) treatment compared with the lower level (2.8 g/tree N). Given that shoot morphology and physiology was similar under high and low N levels and that leaf

Table 2. Plant morphology (plant height, stem diameter, total branches number, total leaf area, and total fresh and dry weight) and physiology [photosynthesis (P_n); stomatal conductance (g_s); and transpiration (E)] of olive transplants grown under different N-source and levels for two growing seasons; 2015 and 2016.

Yr	Treatments	Plant ht (cm)	Stem diam (mm)	Branch no.	Leaf area (cm ²)	Leaf fresh wt (g)	Leaf dry wt (g)	P_n (μmol·m ⁻² ·s ⁻¹)	g_s (mol·m ⁻² ·s ⁻¹)	E (mmol·m ⁻² ·s ⁻¹)
2015	Source (S)									
	Ammonium nitrate	118	11.2	59.4	2,526 a	102.8 a	47.2 a	8.69 ab	0.08	2.74
	Calcium nitrate	118	11.2	53.6	2,109 b	89.3 b	40.9 b	9.16 ab	0.08	2.79
	Osmocote	113	10.8	59.1	2,066 b	84.3 b	38.5 b	9.46 a	0.09	3.07
	Urea	117	11.0	53.8	2,109 b	86.3 b	39.5 b	8.31 b	0.09	2.98
	Level (L)									
	Control	93 b	8.4 b	42.5 b	737 b	31.0 b	13.5 b	6.03 b	0.08	2.47
	2.8 g/tree	121 a	11.4 a	53.5 a	2,178 a	90.1 a	41.3 a	8.67 a	0.08	2.72
	5.6 g/tree	112 a	10.7 a	59.4 a	2,226 a	91.3 a	41.8 a	9.14 a	0.09	3.07
	P value									
2016	S	0.700	0.73	0.25	0.002	0.001	0.0006	0.05	0.50	0.62
	L	0.004	<0.0001	0.001	<0.0001	<0.0001	<0.0001	<0.0001	0.19	0.09
	S × L	0.380	0.21	0.17	0.19	0.54	0.39	0.15	0.85	0.57
	Source									
	Ammonium nitrate	127 ab	13.4 a	98.1	4,034 ab	195.9 ab	90.1 ab	12.80	0.11	2.57
	Calcium nitrate	131 a	13.3 a	105.1	4,205 a	210.0 a	99.4 a	12.35	0.10	2.39
	Osmocote	119 b	12.3 b	97.1	3,666 b	175.0 b	80.7 b	13.16	0.11	2.66
	Urea	121 b	12.4 b	102.1	3,652 b	178.4 b	82.2 b	12.50	0.11	2.54
	Level									
	Control	89 b	8.4 b	54.5 b	916 b	45.7 b	20.7 b	9.52 b	0.09	2.25
	2.8 g/tree	125 a	12.6 a	102.8 a	3,868 a	189.4 a	88.1 a	13.07 a	0.11	2.75
	5.6 g/tree	124 a	13.1 a	98.5 a	3,910 a	190.2 a	87.4 a	12.33 a	0.10	2.33
	P value									
	S	0.03	0.01	0.30	0.05	0.04	0.03	0.74	0.61	0.82
	L	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.001	0.10	0.06
	S × L	0.32	0.37	0.05	0.08	0.09	0.07	0.06	0.31	0.18

Values, in columns within each year, followed different letters are significantly different at $P < 0.05$.

Table 3. Leaf and root N% and NO₃-N % of olive cuttings grown under different N-source and levels for two growing seasons; 2015 and 2016.

			N %		NO ₃ -N %	
Yr		Treatments	Leaf	Root	Leaf	Root
2015	Source (S)	Ammonium nitrate	2.61 a	1.49 a	0.025 ab	0.047 a
		Calcium nitrate	2.35 b	1.31 b	0.011 b	0.031 b
		Osmocote	2.22 b	1.20 b	0.038 a	0.034 ab
		Urea	2.62 a	1.31 b	0.023 ab	0.038 ab
	Level (L)	Control	1.36 c	0.79 c	0.015	0.008 b
		2.8 g/tree	2.36 b	1.23 b	0.024	0.030 a
		5.6 g/tree	2.55 a	1.42 a	0.025	0.045 a
	P value	S	<0.0001	0.03	0.03	0.05
		L	<0.0001	<0.0001	0.56	0.04
		S × L	0.08	0.08	0.13	0.31
2016	Source	Ammonium nitrate	1.70 a	1.31 a	0.025	0.023 b
		Calcium nitrate	1.51 ab	1.21 a	0.011	0.041 a
		Osmocote	1.16 c	1.00 b	0.038	0.010 bc
		Urea	1.47 b	1.23 a	0.023	0.019 b
	Level	Control	0.71 c	0.74 c	0.015	0.003 b
		2.8 g/tree	1.34 b	1.08 b	0.024	0.018 a
		5.6 g/tree	1.59 a	1.30 a	0.025	0.029 a
	P value	S	0.002	<0.0001	0.28	0.001
		L	0.0001	<0.0001	0.73	0.02
		S × L	0.06	0.22	0.59	0.59

Values, in columns within each year, followed different letters are significantly different at $P < 0.05$.

Table 4. Total root dry weight, length, surface area (RSA), diameter, volume, and fork number of olive cuttings grown under different N-source and levels for two growing seasons; 2015 and 2016.

		Root components						
Yr	Treatments	Dry wt (g)	Length (cm)	RSA (cm ²)	Diam (mm)	Volume (cm ³)	Fork (no.)	
2015	Source (S)	Ammonium nitrate	12.7	13,238 a	2,494 a	0.63 b	38.7 a	3,936 a
		Calcium nitrate	12.4	9,426 bc	1,938 b	0.67 a	32.6 b	2,919 bc
		Osmocote	12.0	9,353 c	1,842 b	0.65 ab	29.9 b	2,309 c
		Urea	13.9	11,691 ab	2,215 ab	0.63 b	34.5 ab	3,537 ab
	Level (L)	Control	8.6 c	8,652 b	1,689 b	0.66 a	27.7 b	2,085 b
		2.8 g/tree	14.2 a	10,126 ab	2,067 a	0.67 a	34.7 a	2,921 a
		5.6 g/tree	11.3 b	11,728 a	2,177 a	0.62 b	33.1 a	3,429 a
	P value	S	0.40	0.003	0.01	0.007	0.02	0.002
		L	<0.0001	0.004	0.03	<0.0001	0.07	0.005
		S × L	0.34	0.006	0.02	0.21	0.06	0.11
2016	Source	Ammonium nitrate	42.5 a	21,049 a	3,728 a	0.67	75.6	3,809 a
		Calcium nitrate	40.6 ab	20,468 ab	3,635 ab	0.71	75.4	3,694 ab
		Osmocote	37.9 ab	19,677 ab	3,508 ab	0.70	69.9	3,535 ab
		Urea	33.9 b	18,482 b	3,317 b	0.66	69.4	3,296 b
	Level	Control	18.6 b	13,892 b	2,583 b	0.69	55.7 b	2,378 b
		2.8 g/tree	37.4 a	19,528 a	3,484 a	0.71	71.8 a	3,506 a
		5.6 g/tree	40.0 a	20,310 a	3,610 a	0.66	73.3 a	3,662 a
	P value	S	0.04	0.05	0.04	0.39	0.15	0.05
		L	<0.0001	<0.0001	<0.0001	0.16	<0.0001	0.001
		S × L	0.001	0.001	0.001	0.88	0.002	0.01

Values, in columns within each year, followed different letters are significantly different at $P < 0.05$.

N in the lower N level was within the range, 2.8 g/tree N proved to be adequate to maintain a healthy and balanced growth in olive cuttings.

Root components. Root growth requires sufficient supply of nutrients from the soil and translocation of photosynthates from the shoot. Changes in N nutrient availability in the growing medium affects root-hair formation, primary root growth, and lateral root formation (Leskovar and Othman, 2016; López-Bucio et al., 2003). During the study period 2015–16, significant differences between fertilizer sources and rates were found (Table 4; Fig. 2). Ammonium nitrate and urea N fertilizer sources enhanced root components as compared with CN and OSC, espe-

cially in 2015. However, root growth components increased with AN, CN, and OSC sources compared with urea at the end of the study period, 2016. The importance of the two N forms contained in fertilizer products was highlighted in a study by Lu et al. (2009) in tomato (*Lycopersicon esculentum*) cuttings. Nitrate form alone at 16 mM reduced total uptake of P, Fe, Mn, and Zn, compared with urea and AN in ‘Kalamon’ olive (Tsabarducas et al., 2017). Nitrogen form can significantly affect root and shoot growth and development (Lu et al., 2009; Takács and Técsi, 1992). Moreover, both N form and NO₃⁻/NH₄⁺ ratios play key roles in shoot and root development and the ultrastructure of chloroplasts in olive (López-

Bucio et al., 2003; Takács and Técsi, 1992). For example, at a 3:1 NO₃⁻/NH₄⁺ ratio the surface area of the photosynthesizing membrane was higher than at 0:1 or 1:3 NO₃⁻/NH₄⁺ ratio (Takács and Técsi, 1992). A homogeneous supply of 3:1 NO₃⁻/NH₄⁺ ratio to tomato transplants resulted in the maximum shoot and root dry matter, root surface area and root volume compared with 1:0, 0:1, or 1:1 NO₃⁻/NH₄⁺ ratios (Lu et al., 2009).

Nitrate and phosphate act as signals regulating root cell division and differentiation, with remarkable effect on the overall root system architecture (López-Bucio et al., 2003). Root N uptake is regulated locally by the concentration of N in the substrate and by a systemic feedback regulated by whole-plant signals of N status (Nacry et al., 2013). Excessive annual applications of N fertilizer may lead to toxic overdose of N and negative impacts on both the plant and the environment (Fernández-Escobar et al., 2014). Tsabarducas et al. (2017) found that NH₄⁺-N at 225 mg·L⁻¹ resulted in lower net photosynthetic rate, total Ca²⁺, Mg²⁺, and higher K uptake compared with lower N levels. Given that most root components were statistically similar at both N levels, we believe that excessive amount of N such as the high level of N (5.6 g/tree N) to olive transplants is unnecessary.

The N source and level treatment interactions revealed that root component (root volume, surface area, and root length) from control (0 g/tree N) plants was higher than 2.8 and 5.6 g/tree N rates of AN and OSC during the first growing season (2015) and significantly lower than both levels at the end of the experiment, July 2016. This is can be attributed to an adequate leaf N concentration in the untreated-control cuttings leaves during the first year (1.3% N) compared with the second year (0.7% N); which was below the N deficiency threshold (1.22% to 1.35% N of dry weight) as suggested by Molina-Soria and Fernández-Escobar (2010) for mature trees. As external inputs of NO₃⁻ and NH₄⁺ levels increased in agricultural soils, elevated rates of NO₃⁻ and NH₄⁺ efflux lower the capacity of roots to uptake external N; except N-starved roots that have a high absorption capacity (Glass, 2003). The delivery of high NH₄⁺ concentration to plant roots actively absorbing NO₃⁻ reduces NO₃⁻ uptake and lowers the efficient utilization of soil NO₃⁻ (Glass, 2003).

Slow-release fertilizers release nutrient to soil slower than conventional fertilizers and the duration of release depends on microbial activity that is driven by soil moisture and temperature conditions (Liu et al., 2017). Slow-release fertilizers have been recommended by environmentalist as an alternative to traditional fertilizer to reduce the toxic effect of excessive N lost by leaching (Fernández-Escobar et al., 2004; López-Bucio et al., 2003; Socolow, 1999). This could be attributed to the fact that slow-release fertilizer can reduce nutrient losses and increase nutrient-use efficiency (Liu et al., 2017). It has been reported that the total N losses from slow-release fertilizers

Fig. 2. Total root length, surface area, volume, total root dry weight, and total fork number of olive cuttings grown under different N-sources and levels for two growing seasons; 2015 and 2016. AN = ammonium nitrate; CN = calcium nitrate; OSC = slow-release Osmocote.

are lower than traditional fertilizers such as, AN and CN (Fernández-Escobar et al., 2004). In this study, the response of olive cuttings to

the controlled-released fertilizer (OSC) levels was different from other studied sources (urea, AN, CN). Although cuttings that

received a lower rate (2.8 g/tree N) of AN, CN and urea showed similar or better growth response than the higher rate (5.6 g/tree N),

olive cuttings that received a higher rate of OSC had better root growth than those treated with the lower rate (Fig. 2). Fernández-Escobar et al. (2004) found that olive 'Picual' seedling fertilized using traditional and slow-release N fertilizers exhibited similar growth rate at a low N rate (0.75 g/tree N) but greater shoot growth was obtained with slow-release N fertilizers when higher level (2.0 g/tree N) of fertilizer was applied.

Conclusions

In this study we found that olive young trees that received 2.8 g/tree N rate had similar plant morphology (plant height, diameter, branch number, leaf area, and fresh weight) and gas exchange (P_n , g_s and E) compared with those that receive 5.6 g/tree N. However, plant morphology and gas exchange was significantly reduced in the absence of N fertilizer (control). Nitrogen source had a significant effect on olive shoot and root morphology, physiology and leaf and root N concentration. It was clear that CN (2.8 g/tree N) was the best in terms of growth of the aerial part and never worse than AN in terms of root growth and structure at the end of the experimental period. Root components treated with high N rate (5.6 g/tree N) using the slow-release fertilizer (OSC) was similar to those treated with the low AN rate (2.8 g/tree N). Therefore, for nursery containerized olive trees of 'Arbequina' or other cultivars with comparable growth rates, we recommend to apply the CN or AN source at 2.8 g/tree N or the controlled-released fertilizer OSC at 5.6 g/tree N.

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