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 (Pₐ), transpiration (E), and stomatal conductance (gₛ)], 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%Ca), ammonium nitrate (AN, 12%N, 17%Ca), 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 (Pₐ, gₛ, 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 (Bousadia 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 (Bousadia 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 (Bousadia et al., 2015). Developmental root components including root hairs and lateral roots are particularly 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 (Bousadia 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,
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°45′21.6″W, long. 29°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, NO$_3^- +$ 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 tree per each in 2016 were 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 deference test ($P = 0.05$) in SAS (Version 9.4 for Windows; SAS Institute, Inc., Cary, NC) was 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 (Pn, gs and E) from the 2.8
Values, in columns within each year, followed different letters are significantly different at P < 0.05.
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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.

| Yr | Treatments | Dry wt (g) | Length (cm) | RSA (cm²) | Diam (mm) | Volume (cm³) | 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 | 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 | 15.9 a | 11,728 ab | 2,177 a | 0.68 a | 33.1 a | 3,429 a |
| P value | S | <0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| S × L | 0.06 | 0.02 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 |

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, especially 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 lower 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 nutrient-use efficiency (Liu et al., 2017). It has been reported that the total N losses from slow-release fertilizers
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 ‘Piciual’ 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, di-ameter, 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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