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Short-term water stress affecting NO_3^- absorption by almond plants☆

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ABSTRACT

Identifying the rates of nitrate absorption by almond plants under water stress is important for improving nitrogen use efficiency in almond orchards. This study aimed to determine the rates of nitrate absorption by almond plants subjected to short-term water stress and no water stress, and the kinetic NO_3^- absorption parameters in well-watered three-year-old almond plants. Two assays were performed. In the first, fourteen plants arranged in completely randomized design received nutrient solutions with water potentials (Ψ_w) of 0.0, −0.2, −0.4 and −0.7 MPa and containing 1.2 mmol l^{-1} KNO_3. We accessed the NO_3^- depletion in these solutions over an 8-h interval and estimated regression curves of the quantity of nitrate in the nutrient solution (Q) as a function of time (t). Subsequently nitrate uptake rates were calculated. Water loss; nitrate reductase activity; nitrate and total N of the sap; nitrate concentration of leaf dry matter; and nitrate and nitrite concentration of root dry matter were, either, evaluated. The second assay was performed the same way of the first one, with four well-watered plants submitted to 0.3 mmol l^{-1} KNO_3 as initial concentration, and allowed estimating NO_3^- absorption parameters V_{max}, K_m and C_{min}. We observed a linear decrease in the nitrate content of the containers receiving 1.2 mmol l^{-1} of NO_3^- over a period of 8 h. In this concentration, the rates of NO_3^- uptake ranged from 1.11 to 3.43 μmol g^{-1} h^{-1} and decreased in low water potentials at the root medium. Root nitrate reductase activity followed the same trend of nitrate absorption. In the low NO_3^- concentration range, the absorption showed a Michaelis–Menten pattern, being the kinetic parameters of NO_3^- absorption 1.15 ± 0.27 μmol g^{-1} h^{-1}, 28.81 ± 4.12 μmol l^{-1} and 17.93 ± 1.1 μmol l^{-1} for V_{max}, K_m, and C_{min}, respectively. We concluded that NO_3^- uptake of almonds is affected by short periods of water stress, with harmful effects occurring at Ψ_w below −0.18 MPa.

1. Introduction

Efficient fertilization requires careful consideration of plant requirements, expected productivity, mobility of nutrients in the soil and fertilizer characteristics. The low recovery rate of nitrogen, approximately 60%, in both rainfed and irrigated crops is a major technical challenge of nitrogen fertilization. To optimize the efficiency of plant acquisition and use of nutrients, it is essential to provide nutrients at optimal concentrations that coincide with the maximum capacity of the roots to absorb them.

Knowing the absorption rates of nutrients by plants is a prerequisite for improving fertilization practices. Such knowledge is necessary for optimizing nutrient use efficiency and achieving a higher recovery of nutrients delivered via conventional fertilization or fertigation, especially nitrogen, which is easily leachable in the soil and is a major contributor to the greenhouse effect due to nitrogen loss in the form of N_2O during the denitrification process.

Schellemberg et al. (2012) measured N_2O emissions in irrigated almond orchards where nitrogen fertilizers (ammonium and nitrate) were applied post-harvest (spring and summer) at different rates. They found that the maximum rates of N_2O emission occurred 24 h after fertilization in the summer season. The emission rates

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were also positively correlated with ammonium and nitrate levels in the soil. They concluded that to mitigate global warming, more efficient irrigation and fertilization strategies are needed.

To optimize nutrient recovery rates and to prevent environmental damage, it is essential to supply nutrients in adequate concentrations at the time and place at which maximum root growth and nutrient absorption occur.

Despite the importance of nutrient uptake for plant growth and survival, little is known about the rates of nutrient uptake by the roots of trees (Lucash et al., 2007). Nutrient absorption rates in general, and N absorption in particular, are well documented for short cycle species such as corn and barley (Kochian and Lucas, 1982; Siddqi et al., 1990). For tree species, most of the nutrient absorption studies (nutrient uptake/root mass), including the absorption of nitrogen had been made on seedlings in controlled conditions (Kelly and Barber, 1991; Kronzucker et al., 1995; Cerezo et al., 1997; Kronzucker et al., 1997; Lima et al., 2005), which may not represent the absorption rates later, in the plant production cycle.

Orchard crops, including fertigated almond (*Prunus dulcis* (Mill.) D.A. Webb), represent one of the largest uses of N fertilizers in California. California is the main almond producer in the world, accounting for 80% of global almond production (Almond Board of California, 2012). Almond orchards are located in the Central Valley of California and all receive supplemental irrigation and fertilization.

Although fertigation provides water to meet the demands of crops, short periods of water deficit can occur between two fertigation cycles. It has been observed that water stress can alter N mineral nutrition, even if mineral N is fully available at the root surface, resulting in lower N uptake and lower N assimilation (nitrate reductase activity). Furthermore, N allocation to plant organs, water xylem flux and xylem sap concentrations may also be altered (Gonzalez-Dugo et al., 2012).

This study aimed to determine the rates of NO_3^- absorption and nitrate reductase activity in three-year-old almond plants grown under well-watered and water stress conditions. It also aimed to determine V_{max}, K_m and C_{min} of NO_3^- absorption in well-watered three-year-old almond plants.

2. Materials and methods

2.1. Plant material and pre-experimental grown conditions

To reach the outlined objectives twenty almond plants, variety Non Pareil, grafted on Nemaguard rootstock, were selected from a collection maintained under fertigation in a greenhouse of the Plant Science Department at UC Davis, California. Such plants had been grown during three years in 10-l pots containing expanded clay (2:1 mix of Profile Golf and Turface® MVP®) as substrate.

After flowering, the selected plants have received distilled water for a period of eighteen days and then they were pruned to achieve a four branches tree canopy approximately 0.7 m high, and 0.55 m in diameter, and transplanted into 18-l pots containing the same substrate described above. Subsequently the 18-l pots were placed on a bench and attached to a circulating hydroponic system with four 40-l reservoirs. The hydroponic system was built following the system design described by Silva Filho (2011). The plants were arranged over the bench in three rows, spaced of 1.0 m between plants in each row and 0.70 m between rows. Each reservoir pumped distilled water and/or nutrient solution to five plants.

After transplantation, the plants continued receiving only distilled water more 17 days and then began to receive complete nutrient solution containing 5.0, 0.5, 3.23, 2.25, 1.0 and 1.75 mmol l^{-1} N–NO_3^-, P, K, Ca, Mg and S respectively, plus 23, 0.3, 12, 0.3, 1.0 and 40 μmol l^{-1} of B, Cu, Mn, Mo, Zn and Fe, respectively.

The nutrient solution was pumped into the system at 6:00, 9:00, 12:00, 16:00, and 20:00 h. The pH and electrical conductivity (EC) of the nutrient solution were measured daily. Whenever necessary pH was adjusted to 5.5–6.5 by addition of HCl 2 mol l^{-1}, and the EC to 1.1 dS m^{-1} adding concentrated nutrient stock solutions. The plants were cultivated in this system for 26 days.

On May 07, 2013, eighteen plants were selected and carefully removed from the substrate and transferred to plastic 9-l containers containing aerated nutrient solution with the same nutrient concentrations used in the circulating hydroponic system. The pH and nutrient concentrations of the solution were tracked and maintained as previously described.

Twenty two days after, on the morning of May 29, 2013 at 8:00 h the plants received 9-l of nitrogen-free nutrient solution containing 0.20, 0.858, 0.254, 0.20, 0.20 and 1.163 mmol l^{-1} of P, K, Ca, Mg, S and Cl, respectively, plus 4.66, 0.057, 2.433, 0.057, 0.20 and 8.11 μmol l^{-1} of B, Cu, Mn, Mo, Zn and Fe, respectively. Each entire container (container plus solution plus plant) was weighed. Subsequently, the plants received 1/5 of the dose of polyethylene glycol (PEG) 8000 required to reach the following water potentials: 0.0 MPa (eight plants), −0.2 MPa (four plants), −0.4 MPa (three plants) and −0.7 MPa (three plants). The other 4/5 of the dose was added gradually on the following dates: 05/29 (18:00 h), 05/30 (8:00 h), 05/30 (18:00 h) and 05/31 (8:00 h). The doses of PEG 8000 applied were 0, 120, 176 and 239 g l^{-1}. These doses were determined based on a mean solution temperature of 26.5 °C, according to Villela and Beckert (2001).

2.2. Determination of depletion curves

2.2.1. First assay: high NO_3^- external concentration and water potentials

On May 31, 2013, an assay was performed with fourteen plants arranged in a completely randomized design with four treatments, four repetitions of the treatments 0 and −0.2 MPa and three repetitions of the treatments −0.4 and −0.7 MPa. The assay began at 9:00 h, after weighing and reestablishing the initial weigh of each container by adding distilled water. Each container received 1.2 mmol l^{-1} NO_3^- as KNO_3.

The evaluation of the nitrate depletion on the nutrient solution was initiated at 12:00 h, 6 h after sunrise and 3 h after nitrate supplementation. This procedure guaranteed the achievement of the steady state rate of absorption. Twelve samples of the nutrient solution were taken along the day, at 12:00, 12:15, 12:30, 12:45, 13:00, 13:30, 14:00, 14:30, 15:00, 16:00, 18:00 and 20:00 h following the methods described by Claassen and Barber (1974) and Lima et al. (2005). During this period, the pH was periodically measured, and adjusted to 5.5–6.8. The air temperature ranged from 30 to 36.7 °C, and the temperature of the nutrient solution ranged from 23 to 30 °C. Daily global radiation, measured with an Eppley pyranometer, was 755 LY (Atmospheric Science Program, UC Davis, May 2013).

At 17:00 h, four leaf disc samples with a diameter of 5 mm, ranging between 100 and 200 mg fresh weight, were removed from index leaves and stored in liquid nitrogen. At 20:00 h, immediately after pick up of the last nutrient solution samples, approximately 1 g of fine roots of each plant was collected and stored in liquid nitrogen. Then, to estimate water loss from transpiration over the 12 h assay period, each container was reweighed.

Samples of entire index leaves (10 per plant) and fine branches (20 per plant) were collected at 20:00 h. To extract the sap, these branches were rinsed in distilled water, dried with paper towels and placed in 100-ml tubes filled with ethyl ether and stored at −80 °C according to Souza et al. (2012). The samples of leaf disks, fine roots either were transferred to −80 °C prior to determining NR activity and sap nitrate concentration. The sap (a mixture of