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Revisiting nutrient management for *Citrus* production: to what extent does molybdenum affect nitrogen assimilation of trees?

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ABSTRACT

Increasing the nitrogen (N) use efficiency of fruit trees to enhance fruit yield and decrease N rate and fertilization losses in the field is intensively discussed. Noteworthy, molybdenum (Mo) demand is likely to increase in high yielding citrus orchards. However, supply of this micronutrient through fertilization practices is not well-known. Thus, two experiments were carried out under greenhouse conditions to evaluate the nitrate reductase (NRase) activity and the Mo mobility in sweet orange plants (1-yr-old) after foliar application of Mo. For both experiments, the plants were supplied with two N levels via fertigation over 7-mo (totaling 2.8 and 17.5 g of N per plant), with Mo treatments applied in the final month. The first experiment consisted of leaf sprays to the whole plant canopy at 0 (control), 0.12, 0.60 and 1.20 g L^{-1} Mo. In the second experiment, the 0.60 g L^{-1} Mo spray was limited to one side of the canopy. The Mo supply enhanced the NRase activity either in leaves or roots and increased the nitrate uptake by roots. Consequently, the N content in the roots, twigs and leaves of plants increased. When the Mo was sprayed on one side of the canopy, the nutrient was translocated (30 − 40% from the absorbed) from the leaves to the roots, but at a lower percentage in plants grown with the highest N supply. Although the Mo concentration did not increase in leaves that did not directly receive the micronutrient spray, the NRase increased in both parts of the canopy, as well as in the roots, enhancing the N content in *Citrus*.

1. Introduction

Molybdenum (Mo) is a micronutrient that catalyzes a range of reactions, such as phytohormone synthesis, sulfite detoxification, purine degradation and nitrogen (N) assimilation by plants (Schwarz et al., 2009; Bittner, 2014). Molybdenum is a cofactor of nitrate reductase (NRase; E.C. 1.7.1.1), the first enzyme involved in the intracellular reduction of nitrate (NO_3^-) to ammonium (NH_4^+). The *in vivo* NRase activity is used as an indicator of the N assimilation potential of plants (Dovis et al., 2014).

Visual symptoms of Mo deficiency in field grown trees are unusual because the adequate and deficient values of the nutrient in plant tissue are constrained to a narrow range (Obreza and Morgan, 2008; Mattos Jr. et al., 2012). Stewart and Leonard (1952) reported on Mo deficiency of citrus plants in Florida (USA). However, no other reports of visual symptoms of Mo deficiency in tree crops are available in the literature. Furthermore, few experiments have evaluated the effects of Mo fertilization (Ezz and Kobbia, 1999; Srivastava and Singh, 2007), and consequent absorption and mobility of the foliar-applied nutrient. Yet, such information could contribute to practices that foster the maximum fruit yield capacity of plants.

The Mo demand by citrus trees is likely related to several agronomic factors, particularly in tropical soils, where the availability of the nutrient is limited by the high acidity potential of the soil and the low content of this micronutrient in the soil matrix (Kaiser et al., 2005). Among these factors, the increased vigor of the plant, due to intensive N fertilization (Alva et al., 2006; Boaretto et al., 2007; Quiñones et al., 2012) and the utilization of rootstocks with superior nutritional demand (Quaggio et al., 2004; Mattos Jr. et al., 2006) can lead to a greater need for micronutrients, such as Mo, involved in enzymatic processes (Bondada and Syvertsen, 2003; Schwarz and Mendel, 2006; Schwarz et al., 2009).

The recent increase in citrus areas grown under fertigation and the enhancement of NO_3^--containing fertilizer application (Quaggio et al., 2014), favors greater demand for Mo by citrus trees (Kaiser et al., 2005). Groves fertigated with calcium nitrate present improved soil chemical conditions for root development and provide a more balanced

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nutritional status than those fertilized with NH_4NO_3 (Quaggio et al., 2014) because high N doses and continuous application of NH_4^+ sources can cause soil acidification (Britto and Kronzucker, 2002).

Although fertigation with calcium nitrate enhances sweet orange fruit yields (Quaggio et al., 2014), Mo-deficient plants present low NRase activity, what could limit their ability to attain maximum production efficiency. In plants, NO_3^- is reduced to NH_4^+ before it is incorporated into organic forms by two distinct sequential reactions, catalyzed by different enzymes. The first reaction occurs in the cytosol of root and/or leaf cells and is mediated by NRase that reduces NO_3^- to nitrite (NO_2^-) (Lea et al., 1999; Mendel, 2011; Dovis et al., 2014). The NO_2^- formed is then transported to the plastids of roots or chloroplasts of leaves, where it is reduced to NH_4^+ by nitrite reductase (Lea and Azevedo, 2007). The assimilation of inorganic-N into an organic form impacts plant growth and the potential yield of crops (Lea et al., 1999). The supply of Mo improves the N assimilation by plants growing in acid soils (pH 4.5 − 5.0), resulting in improved growth and yield of wheat (Wang et al., 1999) and grapevines (Williams et al., 2004).

Despite the positive correlation between the NRase activity and the infiltration of Mo in leaf fragments (Shaked and Bar-Akiva, 1967), there is still a lack of information about how Mo supply and mobility characteristics in woody trees contribute to increase the enzyme activity of plants grown at different levels of N−NO_3 and this is the purpose of these experiments.

2. Material and methods

Two experiments were carried out sequentially in a greenhouse with similar management practices during the initial stages of plant growth. Homogeneous 1-yr-old sweet oranges plants [*Citrus sinensis* (L.) Osbeck *cv*. Valencia] grafted onto Rangpur lime (*C. limonia*) were grown in pots containing 20 dm^3 of an organic substrate (80% pinus bark, 15% vermiculite and 5% of carbonized material; pH 5.6).

2.1. Experiment 1: NRase activity in citrus plants after foliar application of Mo

The first experiment was set up in a 4 × 2 factorial design with four doses of Mo (nil application, 0.12, 0.60 and 1.20 g L^{-1}) and two levels of N, with four replicates. Plants were divided into two groups that received different levels of N to impose contrasting nutrient status based on the expected demand of the plants. Then a fertilization regime was conducted every 15 d via fertigation, which totaled 2.8 and 17.5 g of N per plant, as $Ca(NO_3)_2.4H_2O$. Plants that received the lowest N fertilization level were also supplied with $CaCl_2$ to standardize the amount of Ca against those with the highest N level.

During the experiment, the plant fertilization maintenance was conducted fortnightly according to Hippler et al. (2015), with the following modifications: 250 mg L^{-1} of KH_2PO_4, 493 mg L^{-1} of $MgSO_4{\cdot}7H_2O$, 3.0 mg L^{-1} of H_3BO_3, 2.0 mg L^{-1} of $CuSO_4{\cdot}5H_2O$, 4.0 mg L^{-1} of $ZnSO_4{\cdot}7H_2O$ and 2.0 mg L^{-1} of $MnSO_4{\cdot}3H_2O$. Overall, the experiment received a total of 7.5 g of P, 9.7 g of K, 29 g of Ca, 7.0 g of Mg, 9.4 g of S, 109 mg of B, 82 mg of Cu, 168 mg of Zn and 110 mg of Mn. Iron was applied separately every month, as Fe-EDDHA (iron salt of ethylenediamine-di-*o*-hydroxyphenylacetic acid), in a solution with 0.06 g L^{-1} of Fe.

Molybdenum doses were sprayed on the leaves in a single application 180 d after starting the different N levels, when trees presented the second vegetative flush already mature. The pots were covered with a plastic film to avoid contamination of the substrate with the micronutrient. The amounts of solution retained on the canopy ($Mo_{retained}$) were calculated according to Eq. (1). The volume of sprayed solution remaining on every plant canopy was 40 ± 2.6 mL, which totaled 7.8 ± 2.0 mg of Mo per plant when sprayed with 0.12 g L^{-1} of Mo solution; 39 ± 2.9 mg of Mo per plant with 0.60 g L^{-1} of Mo; and 78 ± 4.5 mg of Mo per plant with 1.20 g L^{-1} of Mo.

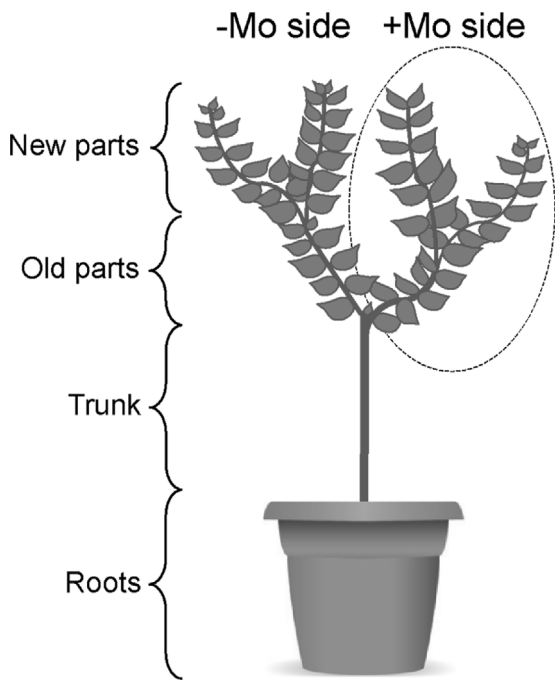

Fig. 1. Diagram of foliar spray of molybdenum (Mo) in Experiment 2. Plants conducted to grow two scaffold branches to which 0.60 g L^{-1} of Mo was sprayed to one side of the canopy (+Mo side; as outlined) or not (-Mo side).

$$Mo_{retained} = [(\text{plant weight after Mo spray}) - (\text{plant weight before Mo spray})] \times (\text{Mo solution concentration}) \quad (1)$$

The *in vivo* NRase activity assay (Dovis et al., 2014) was performed on leaves from the second vegetative flush (new parts; Fig. 1) and fine roots (< 3.0 mm ø) at 1, 4, 9, 15 and 30 d after the Mo application. Briefly, 200 mg fresh weight (FW) of leaves or 1000 mg of FW of fine roots were incubated in 100 mM sodium phosphate buffer solution (pH 7.5) with 200 mM KNO_3 and 1% *n*-propanol (w/v). The samples were vacuum filtered and kept in the dark at 40 °C for 30 min. The NO_2^- was quantified by absorbance at 540 nm, with the addition of 1% sulfanilamide solution in 2.4 N HCl + 0.02% *N*-[1-naphthyl] ethylenediamine dihydrochloride (NED; w/v). The NRase activity was transformed to relative activity considering the treatment with the lowest N supply and without Mo application as the control (100%).

Before NRase activity measurements were determined, the chlorophyll index (SPAD value) was indirectly measured using an SPAD-502 portable meter (Konica Minolta Holding Inc., Tokyo, Japan).

At 30 d after the Mo application, the plants of both experiments were destructively harvested and separated into old and new leaves (Fig. 1), twigs, trunk and roots. The plant parts were washed in detergent at 0.01% (v/v) and deionized water, and dried at 58 − 60 °C till constant weight to quantify the dry mass (DM) production.

The Mo concentration in the plant tissue was determined according to Bataglia et al. (1983), with modifications. One gram of tissue DM was mixed in 6 mL of perchloric acid and maintained for 14 h. Then, 3 mL of perchloric acid was added to the mixture and heated to 220 °C in a digestion block. The sample readings were acquired by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin-Elmer, 5100 PC, Norwalk, CT, USA). A reference material was used as a standard in all sets of analyses. The plant tissue N concentration was quantified according to Bataglia et al. (1983), while NO_3^- and NH_4^+ were determined by steam distillation according to Tedesco et al. (1995).

2.2. Experiment 2: Mo mobility in citrus plants

Both experiments were conducted under equivalent conditions. Nevertheless, in the second experiment, the foliar application of Mo