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Foliar application of molybdenum improves nitrogen uptake and yield of sunflower

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Molybdenum (Mo) foliar spray may improve the nitrogen acquisition by the plants and increase the crop yield. The effects of Mo foliar spray on nitrogen nutrition, achene yield and yield components of sunflower were investigate in this study. The experiment was carried out on a Rhodic Hapludox in Chavantes, São Paulo, Brazil under conventional tillage system. Treatments consisted of five Mo concentrations [0 (control), 26, 52, 78 and 104 g ha^{-1} of Mo] applied as foliar spray at the growing stage of eight developed leaves – V8 (18 days after plants emergence). Foliar application of Mo rates reduced the nitrate (NO_3^-) concentration, and increased the concentrations of ammonium (NH_4^+), total nitrogen (N) and Mo in the leaf tissue of sunflower. Molybdenum foliar spray did not affect the plant height, stem diameter, capitulum diameter and number of achenes per capitulum of sunflower. Application of 58 and 68 g ha^{-1} of Mo resulted in increased of the thousand achenes mass (40%) and achene yield (27%) of sunflower, respectively, compared to the control. Molybdenum foliar spray improves the nitrogen nutrition and the achenes mass resulting in the increased achene yield of sunflower. Results suggest that Mo deficiency can compromise the nitrogen metabolism of plants, and result in lower achene yield of sunflower.

Key words: *Helianthus annuus* L., molybdoenzymes, nitrate assimilation, plant nutrition.

INTRODUCTION

Molybdenum (Mo) is an important micronutrient for plant growth and occurs in several enzymes catalyzing diverse oxidation–reduction reactions in plants (Mengel and Kirkby, 2001). Molybdenum is component of the nitrate reductase, nitrogenase, xanthine dehydrogenase, aldehyde oxidase, and sulfite oxidase enzymes. Because of its involvement in the nitrate assimilation, nitrogen fixation processes, and transport of nitrogen compounds in plants, molybdenum plays a crucial role in nitrogen metabolism of plants (Li et al., 2013).

Molybdenum normally occurs in soil solution as molybdate ion (MoO_4^{2-}) (Mengel and Kirkby, 2001). Molybdenum deficiency can occur in very weathered soils due to continuous cropping, soil erosion, reduction of soil organic matter, and adsorption by Fe hydrous oxides and hydroxides particularly in acid soils at low pH (Kaiser et al., 2005). Significant increases on grain yield with foliar application of Mo have been reported in several soils of Brazil, with pH values below 5.2 (Škarpa et al., 2013; Zoz et al., 2012; Dourado Neto, et al., 2012; Valenciano et al.,

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2011; Biscaro et al., 2011; Calonego et al., 2010; Ascoli et al., 2008). The Mo availability increases with increasing soil pH and acid soils with pH less than 5.2, the amount of available Mo to plants is extremely low, i.e., 0.10 to 0.25 mg kg^{-1} (Mengel and Kirkby, 2001). Since Mo is highly mobile in the xylem and phloem, and crops require low amounts, this micronutrient can be provided by seed treatments and/or foliar spray. Molybdenum foliar sprays are often more effective than soil applications, particularly for acid soils, and are most effective if applied at early stages of plant development (Valenciano et al., 2011). Effectiveness of foliar spray depends on the nutrient uptake rate by the leaves and its translocation into the plant (Boaretto et al., 2003). The leaves rapidly absorb Mo applied by leaf spray. Campo and Hungria (2002) found that translocation of Mo to the nodules of soybean plants was very rapid, and reported the highest concentration of this nutrient in the nodules five days after application.

The effect of Mo fertilization on increasing plant yield is often related to an increased ability of the plant to utilize nitrogen (N). Biscaro et al. (2011) verified that nitrogen fertilization increased common bean grain yield only when combined with Mo leaf supply. The activities of nitrogenase and nitrate reductase are affected by the Mo status of plants, and their activities are often suppressed in plants suffering from Mo deficiency (Toledo et al., 2010). Molybdenum foliar spray (40 g ha^{-1} of Mo) at 25 days after plant emergence greatly enhanced the nitrogenase and nitrate reductase activities, resulting in increase of total N accumulated in the plant shoots of common bean (*Phaseolus vulgaris* L.) (Vieira et al., 1998). Calonego et al. (2010) found that the absence of Mo foliar supply promoted the accumulation of nitrate in the common bean leaves as result of the increased N availability in the soil, indicating the low efficiency of N assimilation of plants in the absence of this micronutrient. However, the effect of Mo foliar spray on nitrogen acquisition and achene yield of sunflower (*Helianthus annuus* L.) are still unknown.

Sunflower is an annual multi-purpose crop and it has great potential to become an economically important crop in the Brazil considering its use for food, both for animals and humans, fodder plant, and for oil, medicinal and industrial uses. This study investigated the effect of molybdenum foliar spray on nitrogen nutrition, achene yield and yield components of sunflower.

MATERIALS AND METHODS

The experiment was carried out in Chavantes, São Paulo, Brazil (23°04' S, 49°48' W, altitude of 510 m). The soil was a clayey Rhodic Hapludox (Red Latosol in the Brazilian classification), with 360 g kg^{-1} of clay, 130 g kg^{-1} of silt, and 510 g kg^{-1} of sand. Before starting the experiment, soil samples were taken from the surface layer (0 to 0.20 m), air-dried, sieved through a 2.0 mm mesh, and analyzed as in Raij et al. (2001). Soil chemical analysis showed pH in $CaCl_2$ 0.01 mol L^{-1} of 4.8, 18 g dm^{-3} of organic matter, 17 mg dm^{-3} of P (Resin), 7 mg dm^{-3} of S-SO_4, 1.9 $mmol_c$ dm^{-3} of K, 18 $mmol_c$ dm^{-3} of Ca, 7 $mmol_c$ dm^{-3} of Mg, 1.8 mg dm^{-3} of Cu (DTPA), 1.2 mg dm^{-3} of Zn (DTPA), 22 mg dm^{-3} of Fe (DTPA), 14 mg dm^{-3} of Mn (DTPA), 0.45 mg dm^{-3} of B (Hot water) and 0.21 mg dm^{-3} of Mo (1.0 mol L^{-1} ammonium acetate). The area has been cultivated in conventional tillage with soybean in the summer and corn in the fall/winter period.

The regional climate, according to Köppen classification, is Cwa (humid subtropical climate with dry winter and warm summer). The 30-year mean annual temperature is 20.8°C with July minimum of 10.7°C and January maximum of 30.2°C, and mean annual rainfall of 1,420 mm. The experimental design was randomized blocks with five treatments and four replications. Different concentrations of molybdenum [0 (control), 26, 52, 78 and 104 g ha^{-1} of Mo] were applied as a foliar spray at the growing stage of eight developed leaves – V8 (18 days after plants emergence). The Mo source used was Nutry® Molibdênio foliar fertilizer [210 g L^{-1} of Mo and 790 g L^{-1} of inert material, with 1,400 g L^{-1} density]. Molybdenum concentrations were defined according to the recommended product application to the sunflower crop (52 g ha^{-1} of Mo; that is, 250 mL ha^{-1} of commercial product). Applications were performed with a CO_2 pressurized sprayer with 150 kPa working pressure capacity, equipped with flat fan nozzle, adjusted to apply 180 L ha^{-1} broth. The plants were sprayed at dusk due to a lower likelihood of drift by wind speed reduction and higher relative humidity. After spray, a minimum period of 72 h without rain was observed, enabling the best use of the product. Each experimental plot consisted of eight 5.0 m long rows, considering the four central lines as floor area, ignoring 1.0 m from the ends of each row.

Sunflower [*Helianthus annuus* L., Syn 042 hybrid] was sown on October 28, 2013, in rows 0.70 m apart at a density of 4 seeds m^{-1}. Fertilization was carried out by applying 300 kg ha^{-1} 08-24-12 formulation at sowing and 200 kg ha^{-1} 20-00-20 formulation topdressing at the in the growing stage of eighteen developed leaves – V18 (30 days after plants emergence). Pests and diseases control was carried out with two applications of thiamethoxam + lambda-cyhalothrin (ENGEO™ PLENO) and azoxystrobin + cyproconazole (PRIORI XTRA®) at 56.4 g a.i. ha^{-1} + 42.4 g a.i. ha^{-1} and 50 g a.i. ha^{-1} + 20 g a.i. ha^{-1}, respectively.

The concentrations of nitrate (NO_3^-), ammonium (NH_4^+), total nitrogen (N) and molybdenum (Mo) in the leaf tissue of sunflower were determined in developmental stage R4 (at beginning of flowering). Fifteen mature leaves were collected from each experimental unit. The samples were dried in a forced-air oven at 55°C for three days, ground in a Willey type mill and submitted to chemical analysis procedures, as previously described by Malavolta et al. (1997).

Sunflower was harvested when it reached physiological ripeness (stage R9). Agronomic characteristics of the crop were assessed against the following variables: plant height, stem diameter, capitulum (flowerhead) diameter, number of achene per capitulum, thousand-achene mass, and achene yield corrected to 130 g kg^{-1} water content. Data were submitted to analysis of variance and regression, both at the 0.05 level of confidence. The significant equations with the higher coefficient of determination were adjusted. All analyses were performed using SigmaPlot 11.0 software for Windows (Systat Software, Inc., San Jose, CA, USA).

RESULTS AND DISCUSSION

Molybdenum foliar spray affect the nitrate (NO_3^-), ammonium (NH_4^+), total nitrogen and molybdenum concentrations in the leaf tissue of sunflower (Figure 1). Nitrate concentrations in the sunflower leaves decreased progressively with increasing Mo rates in the foliar spray

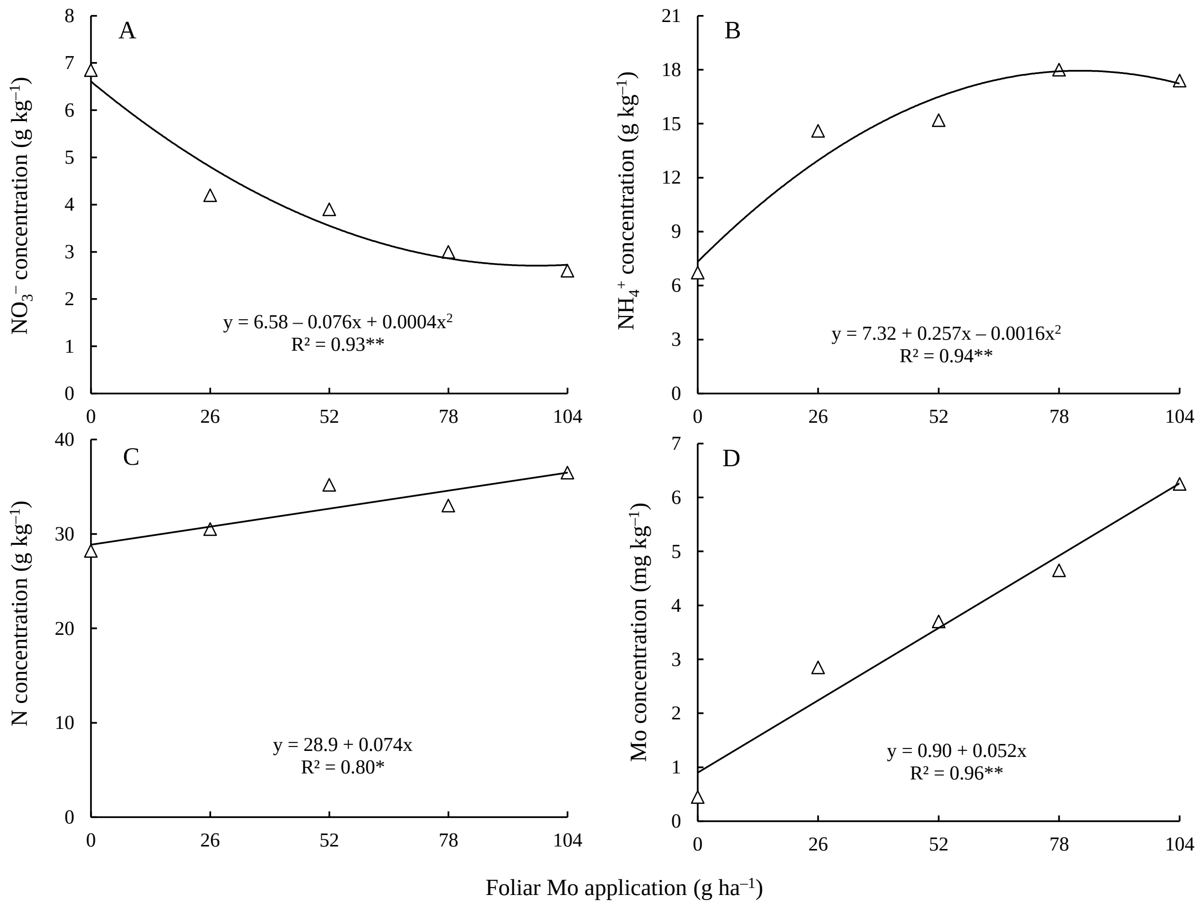

Figure 1. Effect of molybdenum foliar spray on concentrations of nitrate (A), ammonium (B), total nitrogen (C) and molybdenum (D) in the leaf tissue of sunflower (*Helianthus annuus* L.). * and **: statistical significance at 5 and 1%, respectively.

(Figure 1A). Nitrate reduced from 6.58 g kg^{-1} in the control treatment to minimum of 2.97 g kg^{-1} with the application of 95 g ha^{-1} of Mo, indicating mean reduction of 55%. This reduction of nitrate concentration in leaf tissue of sunflower with Mo rates occurred because this micronutrient plays an indispensable role in the nitrate assimilation taken up by plants. Molybdenum is component (cofactor) the nitrate reductase – enzyme that catalyzes the conversion of inorganic N in form of nitrate to nitrite.

The taken up NO_3^- by roots is readily mobile in plants and can be accumulated in vacuoles; however, for nitrate to be used in the synthesis of proteins and other organic compounds in plants, it must be reduced to ammonium (NH_4^+). The reduction is catalyzed by enzymes in two steps: the first step takes place in the cytoplasm by nitrate reductase (NR) transforming NO_3^- into nitrito (NO_2^-), and the second occurs in chloroplasts (shoots) or proplastids (roots) by nitrite reductase (NiR) converting NO_2^- to NH_4^+ (Rosales et al., 2011). The NO_3^- reduction to NO_2^- is the rate-limiting step for primary nitrate assimilation, and reductive ratios in roots and shoots depend on plant species, carbohydrates in plants, and nitrate reductase activity (NRA) as well as environmental conditions such as NO_3^- concentration, medium soil pH, complementary ions, light, and ambient CO_2 concentration (Li et al., 2013). Vieira et al. (1998) showed that the Mo foliar spray enhanced the nitrogenase and nitrate reductase activities resulting in increase of nitrogen accumulated in the common bean leaves. Calonego et al. (2010) reported that the absence of Mo supply promoted the nitrate accumulation in the common bean leaves, indicating the low efficiency of N assimilation by the plants in the absence of this micronutrient.

Molybdenum foliar spray resulted in the significant increase of NH_4^+ and total N concentrations in the sunflower leaves (Figure 1B and C, respectively). Ammonium concentration increased from 28.9 g kg^{-1} in the control to maximum of 36.6 g kg^{-1} with the application of 104 g ha^{-1} of Mo, indicating mean increase of 27%. Total N concentration increased from 7.32 g kg^{-1} in the

Table 1. Effect of molybdenum foliar spray on plant height, stem diameter, capitulum diameter and number of achenes per capitulum of sunflower (*Helianthus annuus* L.).

Foliar application of Mo (g ha^{-1})	Plant height (m)	Stem diameter (mm)	Capitulum diameter (cm)	Number of achene per capitulum
0	1.42	21.0	16.4	589
26	1.50	22.6	17.1	624
52	1.52	24.2	17.4	631
78	1.51	23.4	18.0	602
104	1.54	23.0	17.3	616
Mean	1.50	22.8	17.2	612
F test	2.04ns	0.10ns	1.74ns	1.22ns
Regression	ns	ns	ns	ns
CV (%)	13.1	8.8	7.1	6.8

ns: not significant. CV: coefficient of variation.

control treatment to maximum of 17.7 g kg^{-1} with the application of 80 g ha^{-1} of Mo, indicating mean increase of 142%. The increase of NH_4^+ and N concentration in leaf tissue of sunflower with Mo rates suggests that this micronutrient improved the plant nitrogen assimilation, that is, the NO_3^- reduction to NH_4^+ and subsequently converted in amino acids, proteins and other organic compounds in plants. All inorganic N taken up by plants is first reduced to NH_4^+, because it is the only reduced N form available to plants for assimilation into N-carrying amino acids (Ruiz et al., 2007).

Ammonium is then assimilated into glutamine and glutamate, which serve to translocate organic N from sources to sinks in legumes and non-legumes (Mokhele et al., 2011). The main enzymes involved are glutamate synthase (GS), or glutamine-2-oxoglutarate amino transferase (GOGAT), and glutamate dehydrogenase (GDH) (Wickert et al. 2007; Mokhele et al., 2011). Cao et al. (2008) found that GS has a vital role in NH_4^+ assimilation and the activity of the enzyme is considered critical and possibly rate-limiting step in NH_4^+ assimilation. Ammonium may be toxic to plants, because it can cause proton extrusion, which is associated with NH_4^+ uptake, changes in cytosolic pH and uncoupling of photophosphorylation in plants (Wang et al. 2007). Glutamine and asparagine are the preferential form in which N is assimilated and translocated (Mokhele et al., 2011). This is because these molecules show low C:N ratio, which represents an advantage for NH_4^+ incorporation to non-toxic forms (Frechilla et al., 2002).

Molybdenum concentration on the sunflower leaves increased with increasing Mo rates in the foliar spray (Figure 1D). Molybdenum concentration increased from 0.9 mg kg^{-1} in the control treatment to maximum of 6.3 mg kg^{-1} with the application of 104 g ha^{-1} of Mo, indicating mean increase of 600%. These results were expected due to the low availability of Mo in the soil and its application on the leaves. Molybdenum concentration between 0.6 and 10 mg kg^{-1} is considered adequate for normal growth of plants. Deficient plants shows leaf concentrations between 0.01 and 0.6 mg kg^{-1} (Malavolta et al., 1997). Regardless of the rate applied the Mo concentration in the sunflower leaves remained in the range considered adequate for optimum growth and development of plants. Molybdenum foliar spray did not affect the plant height, stem diameter, capitulum diameter and number of achenes per capitulum of sunflower (Table 1). These agronomic characteristics are predominantly determined by genetic factors, intrinsic to species or cultivar, being little affected by environmental or management factors.

The thousand achenes mass and achene yield of sunflower increased significantly with Mo foliar spray (Figure 2). The thousand achenes mass increased from 59.6 g in the control treatment to a maximum of 83.6 g with the application of 58 g ha^{-1} of Mo, indicating mean increase of 40% (Figure 2A). Based on the magnitude of direct and indirect effects determined by the path analyzes, Rigon et al. (2014) concluded that capitulum diameter and thousand achenes mass are the yield components that determine the achene yield of sunflower. Such inference reports the importance of the achenes mass for increasing the yield of sunflower. The increase in mass of achenes with Mo rates may be due to the increase in N assimilation and nutrition of plants (Figure 1C). The improvement in the N assimilation resulted in a greater amount of assimilates such as glutamine, glutamate and amino acids that were subsequently translocated to the achenes.

The achene yield of sunflower increased from 1,580 kg ha^{-1} in the control treatment to a maximum of 2,002 kg ha^{-1} with the application of 68 g ha^{-1} Mo, indicating mean increase of 27% (Figure 2B). Similar results were obtained by Lima et al. (1999), who found that the application of 75 g ha^{-1} Mo increased the common beans yield. Valentini et al. (2005) reported increase of 43% in the grain yield of maize with the application of 90 g ha^{-1} Mo. Foliar application of Mo up to a dose of 35 g ha^{-1}

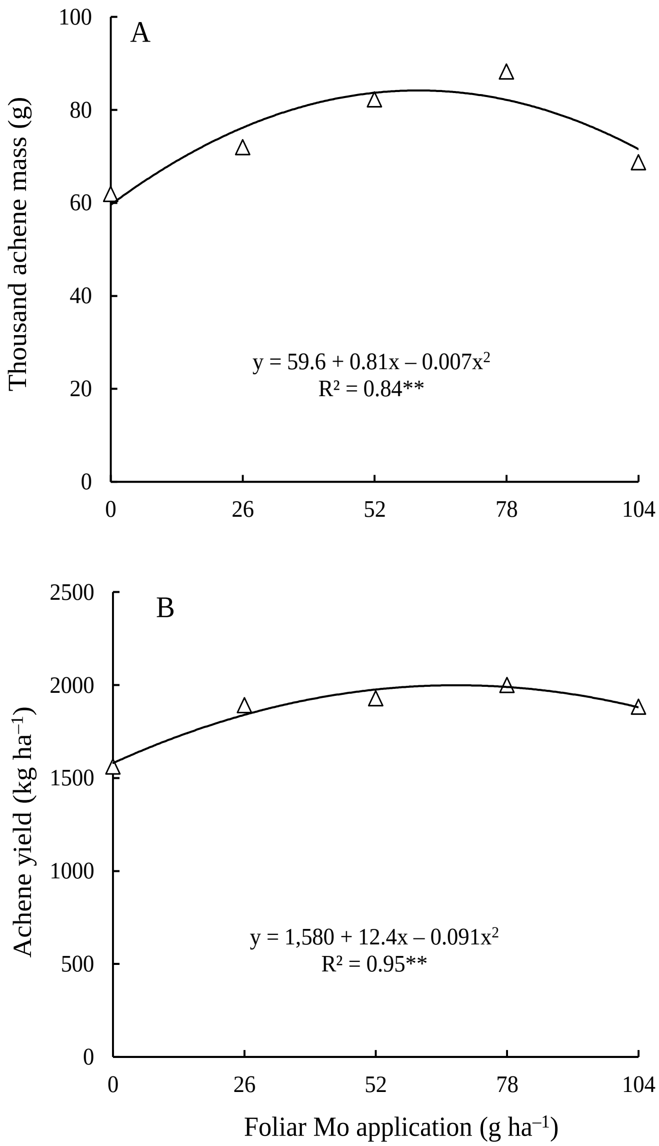

Figure 2. Effect of molybdenum foliar spray on thousand achenes mass (A) and achene yield (B) of sunflower (*Helianthus annuus* L.) **: statistical significance at 1%. CV: coefficient of variation.

increased the yield of wheat (Zoz et al., 2012). Škarpa et al. (2013) found that foliar application of Mo up to a dose of 125 g ha^{-1} at the beginning of vegetation (stage V-4) and developmental stage R-1 increased of achene yields of sunflower.

The effect of Mo fertilization on increasing grain yield is associated with increased ability of the plant to utilize N. Biscaro et al. (2011) verified that nitrogen fertilization increased common bean grain yield only when combined with Mo foliar supply. In general, the results presented here suggest that molybdenum deficiency can compromise the nitrogen metabolism of plants, and result in lower achene yield of sunflower.

Conclusions

Molybdenum foliar spray up to rates of 60 to 70 g ha^{-1} Mo improves the nitrogen nutrition of the plants and the achenes mass of sunflower resulting in the increased achene yield.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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