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Foliar application of nitrogen and molybdenum in sugarcane

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Thesis presented to obtain the degree of Doctor in Science. Area: Soil and Plant Nutrition

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versão revisada de acordo com a Resolução CoPGr 6018 de 2011

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SUMMARY

RESUMO

Adubação foliar com nitrogênio e molibdênio em cana-de-açúcar

O manejo do nitrogênio (N) impacta diretamente o balanço de carbono na produção de bioenergia a partir da cana-de-açúcar. Como forma de aumentar a eficiência da adubação nitrogenada, têm sido adotada a adubação foliar com N, em conjunto com molibdênio (Mo), que pode favorecer a assimilação do N pela participação na redutase do nitrato. A hipótese desta pesquisa é que a adubação foliar com N, associada ao Mo, aumenta a eficiência do uso do N (EUN) e a produtividade da cana-de-açúcar. Foram realizados dois experimentos em solução nutritiva e também experimentos no campo. O primeiro experimento em solução nutritiva avaliou a aplicação foliar de N-ureia em plantas com diferentes suprimentos de N (0,75; 1,5; 3,0; 7,5 e 15,0 mM de N). A adubação foliar com N aumentou a massa seca das folhas e o conteúdo total de N em 128% somente nas plantas supridas com 3,0 mM de N e que apresentaram maior desenvolvimento no momento da aplicação. O segundo experimento em solução nutritiva avaliou a recuperação do ^{15}N aplicado via foliar em plantas com deficiências ou não de N. A aplicação foliar de N aumentou 5,1% a biomassa seca, porém não foi observada interação com Mo. A recuperação do ^{15}N aplicado via foliar foi de 29,5 e 36%, respectivamente, para plantas deficientes e com suprimento adequado de N. Por fim, foram instalados cinco experimentos de campo, avaliados por duas safas agrícolas, totalizando 10 colheitas, avaliando doses de N e Mo aplicados via foliar no estádio de máximo desenvolvimento (dezembro-janeiro). Na safra sujeita a déficit hídrico, a adubação foliar com N e Mo influenciou pouco a produtividade da cana-de-açúcar. Sob condições hídricas adequadas, na média de nove locais a aplicação de 5 kg ha^{-1} N aumentou a produtividade da cana-de-açúcar em 6 t ha^{-1}. As doses de 10 e 20 kg ha^{-1} não promoveram aumento da produtividade da cana-de-açúcar. O Mo contribuiu pouco para o aumento de produtividade, embora tenha aumentado o conteúdo de açúcar da planta no ano seco. A adubação foliar com N tem potencial de aumentar a produtividade da cana-de-açúcar em condições de campo, embora a associação de Mo com o N-ureia foi pouco efetiva em melhorar a recuperação do N-ureia e a produtividade da cana-de-açúcar.

Palavra-chave: *Saccharum* spp., Nutrição, **U**reia

ABSTRACT

Foliar application of nitrogen and molybdenum in sugarcane

Nitrogen (N) management directly impacts the carbon balance in the production of bioenergy from sugarcane. To increase the efficiency of nitrogen fertilization, foliar fertilization with N has been adopted, together with molybdenum (Mo), which can favor N assimilation by participating in nitrate reductase. The hypothesis of this research is that foliar fertilization with N, associated with Mo, increases N use efficiency (EUN) and sugarcane yield. Two experiments were carried out in nutrient solution and also experiments in the field. The first experiment in nutrient solution evaluated foliar application of N-urea in plants with different N supplies (0.75; 1.5; 3.0; 7.5 and 15.0 mM of N). The foliar fertilization with N increased the dry mass of the leaves and the total N content by 128% only in the plants supplied with 3.0 mM of N and that presented greater development at the time of application. The second experiment in nutrient solution evaluated the recovery of ^{15}N foliar applied in plants with or without N deficiencies. The foliar application of N increased the dry biomass by 5.1%, but no interaction with Mo was observed. The recovery of ^{15}N foliar applied application was 29.5 and 36%, respectively, for deficient plants and those with an adequate supply of N. Finally, five field experiments were installed, evaluated by two agricultural seasons, totaling 10 harvests, evaluating rates of N and Mo applied via foliar application at the maximum development stage (December-January). In the crop subject to water deficit, foliar fertilization with N and Mo had little influence on sugarcane yield. Under adequate water conditions, in the average of five locations, the application of 5 kg ha^{-1} N increased sugarcane productivity by 6 Mg ha^{-1}. The rates of 10 and 20 kg ha^{-1} did not promote an increase in sugarcane yield. Mo contributed little to the increase in yield, although it increased the sugar content of the plant in the dry year. Foliar fertilization with N has the potential to increase sugarcane productivity under field conditions, although the association of Mo with N-urea was not very effective in improving N-urea recovery and sugarcane yield.

Keywords: *Saccharum* spp., Foliar nutrition, Urea

INTRODUCTION

In the agreement signed during COP-21 in Paris, Brazil intended to reduce greenhouse gas emissions by 43% by 2030, compared to 2005 levels. Ethanol production should reach 54 billion liters in 2030, almost twice the current production. Sugar production is expected to increase from 38.7 million tons to 46.4 million tons by 2030. To meet these demands for ethanol and sugar production, 942 million tons of sugarcane will need be processed by 2030 (CNI, 2017). Ethanol produced from sugarcane has numerous advantages compared to crops such as corn, wheat and beet, the main one being the energy balance of the order of 5 times more positive compared to other sources (Goldemberg, 2007). Other advantages are the lower N fertilizer consumption and associated to a lowerN_2O emission for sugarcane ethanol production compared to corn ethanol production (Otto et al., 2022).

Nitrogen fertilization has the potential to increase yields in several crops, including sugarcane (Silva et al., 2016). However, sugarcane exhibits low nitrogen use efficiency (NUE), recovering on average 26% of the N applied via fertilizers, while 32% is immobilized into the soil organic matter and the remaining showing a potential to be lost by ammonia volatilization, denitrification, and leaching (Otto, et al. 2016). Inadequate management of N fertilization can reduce the mitigation of CO_2 emissions by 30% from the replacement of fossil fuels with sugarcane ethanol (Jaiswal et al., 2017). In the same direction, Crutzen et al. (2008) shown that the carbon balance advantages in replacing fossil fuels by sugarcane-ethanol is severally compromised depending on the amount of N_2O emitted due to improper N management.

Several strategies can be used to increase NUE in agricultural crops. The most promising are adjusting fertilizer-N rates (Otto et al., 2019; Otto et al., 2020; Castro et. al. 2021), split N application (Otto et al., 2020), leaf N application (Castro, 2022), the use of controlled release fertilizers (Villalba et al., 2014; Guelfi, 2017), fertilizer incorporation into the soil (Castro et al., 2016) and urease and nitrification inhibitors usage (Cantarella, 2007; Silva et al., 2017). Identifying responsive and non-responsive sites to N fertilization is also a potential strategy, besides its complexity. Mariano et al. (2017) found 50% of unburnt sugarcane sites being non-responsive to N fertilization in a 15 sites-study. In the review of Otto et al (2016), 24% of the sites were nonresponsive to N, most of them with a historical usage of vinasse, press mud or planting of legume crops in the renovation period. Otto et al (2019) also identified sites receiving vinasse as non-responsive to N. This exemplifies the uncertainty of sugarcane response to N fertilization and shows a promising scenario for optimizing N fertilization of sugarcane fields.

One strategy to increase NUE in sugarcane systems is to reduce the amount of N applied to the soil, subjected to many losses, and increases the foliar N supply, which usually presents a better recovery by the plant. This strategy, despite promising and already being adopted by sugarcane growers, has not received much attention of research in previous years. Several years ago, Trivelin et al. (1988) showed that the efficiency of foliar N absorption in sugarcane is much higher compared to the efficiency of absorption through the soil (Trivelin et al., 1988). More recently, Castro, (2022) found that it is possible to reduce soil nitrogen fertilization using foliar fertilization.

Despite not well documented in the literature, foliar application of N in addition with molybdenum (Mo), gained attention from sugarcane growers and has been used in commercial fields so far. The addition of Mo is sustained by the role of Mo in nitrate reductase activity, responsible for the incorporation of nitrate in nitrite ammonium inside plant tissue (Campbell, 1999). More recently, in addition to N and Mo foliar products, the fertilizer industry launched several products with micronutrients and compounds such as synthetic hormones, amino acids, humic and fulvic acids and seaweed extracts. However, the effect of such products in sugarcane yield is still lacking scientific validation.

The efficiency of foliar N fertilization is affected by several factors, including the nutritional status of the plant, stage of crop development, leaf age, leaf surface properties and climatic conditions at the time of application (Fageria, et al., 2009; Fernandez, Eicherdt, 2009). The nutritional status of other nutrients can also influence the absorption and translocation of leaf-applied N. Ruan and Gerendas (2015) studied the absorption and translocation of leaf-applied N-urea in *Camellia sinensis* L. under N, potassium (K), magnesium (Mg) and sulfur (S) deficiency. Absorption was affected by N and S deficiency, while translocation was affected by N, K and S deficiency, with emphasis on K, which participates in the circulation of malic acid to the roots via the phloem and returns to the shoots with N via xylem in the form of potassium nitrate (Marschner, 2012). It has also been reported that Mg deficiency can inhibit phloem amino acid transport (Cakmak and Kirkby, 2008).

However, there is no study on the effect of leaf N application on plants subjected to micronutrient deficiencies. The assimilation of N in organic compounds depends on a sequence of reactions in which nitrate reductase (soluble molybdoflavoprotein) participates, an enzyme that has Mo in its composition (Campbell, 1999). Furthermore, Mo is an essential element used as a cofactor in more than 40 enzymes, which are: (i) nitrate reductase, catalyzing the assimilation of inorganic N in the form of NO_3^-, (ii) aldehyde oxidase, catalyzing the last step in the synthesis of abscisic acid (ABA), (iii) xanthine dehydrogenase, acting in purine catabolism and stress reactions and (iv) sulfite oxidase, catalyzing sulfite oxidation. Mo is essential for biological nitrogen fixation and is also a cofactor of nitrogenase in bacteria, making it essential for plants. Mo associates with pterins in plants to be active in the catalytic sites of enzymes that contain Mo (Taiz et al., 2017)

A series of experiments carried out recently indicated a significant response of sugarcane to the application of micronutrients in the planting furrow (Mellis et al., 2016). Studying micronutrients supplementation through the soil in eleven sugarcane fields, the same authors observed that zinc (Zn) and Mo were the micronutrients that showed the most significant effects, reaching yield gains of 19 and 12 Mg ha^{-1}, respectively. Based on this study, the new edition of the official recommendation bulletin of São Paulo State, the main producer of sugarcane in Brazil, recommends the application of Mo both at planting and at ratoon stage (Cantarella, 2022). However, the adoption of this recommendation by farmers depends on an effective way of supplying this element in fertilization programs. Foliar application of Mo can be an alternative, considering the high price of the nutrient and the low Mo rates often used. Indeed, foliar application of Mo showed potential to increase sugarcane productivity, possibly due to its favorable effect on N metabolism (Mellis et al., 2016).

Growers are using foliar fertilization on major crops, and new research has shown some benefits. In a study using foliar Mo fertilization in soybeans and maize, Oliveira et al. (2022) noted improvements in grain protein absorption and crop yield. Another study shows that applying foliar nitrogen to soybeans might alter their carbon metabolism and stimulate the plant, resulting in higher yields (Rodrigues, 2021). Although the research was carried out in a greenhouse and no work has been done with foliar fertilization of N and Mo in field settings, Mellis (2022) discovered an improvement in N incorporation with the application of foliar Mo in sugarcane. The hypothesis of this study is that the combined foliar spray of N and Mo increases N use efficiency and sugarcane yield. The objective of this study was to evaluate, both under controlled and field conditions, the effect of foliar-spray N and Mo on mineral nutrition, N metabolism, ^{15}N recovery and sugarcane yield. This thesis was divided in three chapters:

(1) – Absorption and nutritional status of sugarcane subjected to N-urea foliar spray under different nitrogen supply

(2) – Effects of foliar application of nitrogen (^{15}N) and molybdenum on nitrate and ammonium metabolism in sugarcane cultivated under nitrogen supply levels

(3) – Foliar spray with nitrogen and molybdenum in sugarcane under field conditions

References

CAKMAK, I.; KIRKBY, E. A. Role of magnesium in carbon partitioning and alleviating photooxidative damage. **Physiology Plant**, v. 133, 692–704, 2008.

CAMPBELL, M. K.; FARRELL, S. O. **Bioquímica**. São Paulo: Cengage Learning, 2016.

CANTARELLA, H.; QUAGGIO, J. A.; MATTOS JR, D.; BOARETTO, R. M. RAIJ, B. V. Boletim 100: **Recomendações de adubação e calagem para o estado de São Paulo**. Instituto Agronômico de Campinas, 2022, 581p.

CANTARELLA, H.; TRIVELIN, P. C. O.; CONTIN, T. L. M.; DIAS, F. L. F.; ROSSETTO, R.; MARCELINO, R.; COIMBRA, R. B.; QUAGGIO, J. A. Ammonia volatilization from urease inhibitor-treated urea applied to sugarcane trash blankets. **Scientia Agricola,** v. 65, p. 397–401, 2007.

CASTRO, S. G. Q; DECARO, S. T.; FRANCO, H. C. J.; GRAZIANO. P. S.; GARSIDE, A.; MUTTON, M. A. Best Practices of Nitrogen Fertilization Management for Sugarcane Under Green Cane Trash Blanket in Brazil. **Sugar tech**, v. 19, p. 51-56, 2016.

CASTRO, S. G. Q.; MAGALHÃES, P. S. G.; CASTRO, S. A. Q.; KÖLLN, O. T.; FRANCO, H. C. J. Optimizing Nitrogen Fertilizer Rates at Distinct In-season Application Moments in Sugarcane. **International Journal of Plant Production**, v. 1, p. 1-16, 2021.

CASTRO, S. A. Q. **Aproveitamento do N-fertilizante (N-ureia) pela cana-de-açucar aplicado por via foliar no período de máximo crescimento da cultura em complemento à adubação de solo**. 2022, 22p. Tese (doutorado) – Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 2022.

CNI, Confederação Nacional da Indústria. **O setor sucroenergético em 2030: dimensões, investimentos e uma agenda estratégica**. Marcos Fava Neves; Felipe Gerardi; Rafael Bordonal Kalaki; Renata Gali. – Brasília: CNI, 2017.

CONTIN, T. L. M. **Ureia tratada com inibidor da urease NBPT na adubação da cana-de-açúcar colhida sem despalha a fogo**. 2007. 72p. Dissertação (mestrado). Instituto Agronômico de Campinas, Campinas, 2007.

CRUTZEN, P. J.; MOSIER, A. R.; SMITH, K. A.; WINIWARTER, W. N_2O Release from Agrobiofuel Production Negates Global Warming Reduction by Replacing Fossil Fuels. **Atmospheric Chemistry Physics**, v. 8, p. 389-395, 2008.

FAGERIA, N. K.; FILHO, M. P. B.; MOREIRA, A.; GUIMARÃES, C. M. Foliar fertilization of crop plants. **Journal of Plant Nutrition**, v. 32, p. 1044–1064, 2009.

FERNÁNDEZ, V.; EICHERT, T. Uptake of hydrophilic solutes through plant leaves: current state of knowledge and perspectives of foliar fertilization. **Critical Reviews in Plant Science**, 28, 36–68, 2009.

GOLDEMBERG, J. Ethanol for a sustainable energy future. **Science**, v. 315, p. 808–810, 2007.

JAISWAL, D.; DE SOUZA, A. P.; LARSEN, S.; LEBAUER, D. S.; MIGUEZ, F. E.; SPAROVEK, G.; BOLLERO, G.; BUCKERIDGE, M. S.; LONG, S. Brazilian sugarcane ethanol as an expandable green alternative to crude oil use. **Nature Climate Change**, v. 7, p. 788-792, 2017.

MARIANO, E.; OTTO, R.; MONTEZANO, Z. F.; CANTARELLA, H.; TRIVELIN, P. C. O. Soil nitrogen availability indices as predictors of sugarcane nitrogen requirements. **European journal of agronomy**, v. 89, p. 25-37, 2017.

MARSCHNER H. 2012. **Mineral nutrition of higher plants**. Ed. London: Academic Press. 672 p.

MELLIS, E. V.; QUAGGIO, J. A.; BECARI, G. R. G.; TEIXEIRA, L. A. J.; CANTARELLA, H.; DIAS, F. L. F. Effect of micronutrients soil supplementation on sugarcane in different production environments: Cane plant cycle. **Agronomy Journal**, v. 108, p. 2060–2070, 2016.

MELLIS, E. V.; KÖLLN, O. T.; MOREIRA, L. A.; OTTO, R.; FERRAZ-ALMEIDA, R.; RAMOS, L. F.; ANDRADE, R. P.; FRANCO, H. C. J. Molybdenum increases nitrogen use efficiency of sugarcane under limited N supply, **Journal of Plant Nutrition**, v. 45:9, p.1360-1369, 2022.

OLIVEIRA, S. L.; CRUSCIOL, C. A.; RODRIGUES, V. A.; GALERIANI, T. M.; PORTUGAL, J. R.; BOSSOLANI, J. W.; MORETTI, L. G.; CALONEGO, J. C.; CARARELLA, H. Molybdenum foliar fertilization improves photosynthetic metabolism and grain yields of field-grown soybean and maize. **Frontiers in Plant Science, v. 13, p. 1-12, 2022.**

OTTO, R.; CASTRO, S. A. Q.; MARIANO, E., CASTRO, S. G. Q.; FRANCO, H. C. J.; TRIVELIN, P. C. O. "Nitrogen use efficiency for sugarcane-biofuel production: what is next?" **BioEnergy Research**. v. 9, p. 1272-1289, 2016.

OTTO, R.; MARIANO, E.; MULVANEY, R. L; KHAN, S. A.; BOSCHIERO, B. N.; TENELLI, S.; TRIVELIN, P. C. O. Effect of previous soil management on sugarcane response to nitrogen fertilization. **Scientia agricola**, v. 76, p. 72-81, 2019.

OTTO, R.; NETTO, G. J. M. S.; FERRAZ-ALMEIDA, R.; ALTARUGIO, L. M.; FAVARIN, J. L. Multisite response of sugarcane to nitrogen rates and split application under Brazilian field conditions. AGRONOMY JOURNAL, v. 1, p. 1-15, 2020.

OTTO, R.; FERRAZ-ALMEIDA, R.; SANCHES, G. M.; LISBOA, I. P.; CHERUBIN, M. ,R. Nitrogen fertilizer consumption and nitrous oxide emissions associated with ethanol production - A national-scale comparison between Brazilian sugarcane and corn in the United States. **Journal of cleaner production**, v. 350, p. 131482, 2022

RODRIGUES, V. A.; CRUSCIOL, C. A. C.; BOSSOLANI, J. W.; PORTUGAL, J. R.; MORETTI, L. G.; BERNART, L.; VILELA, R. G.; GALERIANI, T.; LOLLATO, R. P. Foliar nitrogen as stimulant fertilization alters carbon metabolism, reactive oxygen species scavenging, and enhances grain yield in a soybean–maize rotation. Crop Science, v. 61, p 2687-3791, 2021.

RUAN, J.; GERENDÁS, J. Absorption of foliar-applied urea-^{15}N and the impact of low nitrogen, potassium, magnesium and sulfur nutritional status in tea (*Camellia sinensis* L.) plants. **Soil Science and Plant Nutrition**, v. 61, p 653-663, 2015.

SILVA, A. G. B.; OTTO, R.; SEQUEIRA, C.; SERMARINI, R. A. Urease Inhibitor NBPT on Ammonia Volatilization and Crop Productivity: A Meta-Analysis. **Agronomy Journal**, v. 109, p. 1-13, 2017.

TAIZ, L.; ZEIGER, E.; MOLLER, I.; MURPHY, A. **Fisiologia e desenvolvimento vegetal**. 6.ed. Porto Alegre: Artmed, 2017. 888 p.

TRIVELIN, P. C. O.; CARVALHO, J. G.; SILVA A. Q.; PRIMAVESI, A. C. P. A.; CAMACHO, E.; EIMORE, I. E.; GUILHERME, M. R. **Adubação foliar de cana-de-açúcar (*Saccharum* spp) absorção e translocação de uréia-N.** Energia Nuclear na Agricultura. Piracicaba, v. 9, p. 52-65, 1988.

ABSORPTION AND NUTRITIONAL STATUS OF SUGARCANE SUBJECTED TO N-UREA FOLIAR SPRAY UNDER DIFFERENT NITROGEN SUPPLY

Abstract

The carbon balance in sugarcane ethanol production can be improved by increasing nitrogen use efficiency (NUE). One of the strategies adopted to increase NUE is foliar fertilization, which has better absorption efficiency compared to soil fertilization. However, the absorption, assimilation, and translocation of nitrogen (N) foliar absorbed depends on the nutritional status of the plant. In order to assess the effectiveness of N-urea applied in the leaves of plants with different N supplies, an experiment in nutrient solution was conducted. The experiment was conducted as a double factorial (5x2) with 5 supplies of N: 0.75, 1.5, 3.0, 7.5 and 15 mM of N (as ammonium nitrate) and two treatments of foliar N, with foliar N (1.5 mg N plant^{-1}) and control. The effects on dry matter and N, phosphorus, potassium, magnesium, calcium, and sulfur contents in the plant compartments were evaluated. The N supply to nutrient solution that generated better nutritional balance and dry mass production was 3.0 mM of N. Plants with N-deficient supply (0.75 and 1.5 mM of N) had limited growth, whereas plants supplied with 7.5 and 15 mM suffered from the excess supply of N. The ratio between root and shoot biomass varied from 1:0.65, in the supply of 0.75 mM of N, to 1:1.85 in the supply of 15 mM of N, demonstrating thar high N supply to the nutrient solution reduced root growth. The application of foliar N, on average of all N supplies, decreased dry mass of all compartments, whereas it increased N content of treated leaves, stems, and roots. Thus, a toxic effect of foliar N application was evidenced, probably due to the high applied rate. However, plants receiving 3.0 mM of N in the nutrient solution, in addition to N foliar spray, showed an increase in the dry mass of treated leaves, as well as an improvement in total N uptake equivalent to 128% in relation to the control. This study indicates thar 3.0 mM of N is the adequate rate for sugarcane cultivation under nutrient solution and that excessive N supply to the leaf can compromise plant growth.

Key words: Foliar fertilization, absorption efficiency, nutrient solution.

1.1 Introduction

Sugarcane has a low nitrogen use efficiency (NUE), typically, 65-80% of the nitrogen fertilizer added to the soil is not utilized by the crop (Meyer et al., 2007; Franco et al., 2011). In order to improve the NUE in sugarcane, several techniques can be used, but the primary ones include fertilizer rate adjustment, fertilizer splitting, leaf application, supplying of complete and balanced nutrition, the use of controlled release fertilizers, fertilizer incorporation, and urease and nitrification inhibitors (Cantarella, 2007, Villalba, et al. 2014, Guelffi, 2017). Foliar fertilization is a technique that has been frequently used in commercial sugarcane fields but has a lack of scientific validation.

Foliar application of nutrients has been the most popular and widely used fertilizer method for fields and crop cultivation since it is thought to be more effective than soil fertilization (Fernández and Eichert 2009). Urea, a water-soluble organic molecule that is easily absorbed by plant leaves and subsequently distributed throughout the plant, is the main N form that is often used for foliar fertilization (Khan et al., 2017). Urea has the advantage of presenting an uncharged molecule that quickly passes through the leaf cuticle (Fernandez and Eichert, 2009).

The nutritional status of plants, the stage of leaf growth, the age of the leaves, the characteristics of the leaf surface, climatic conditions, and the application timing all have an impact on how effective foliar urea applications perform (Fageria et al. 2009; Fernández and Eichert 2009). Numerous investigations have tried to determine the impact of plant N status on foliar N uptake, but the results remain inconclusive. A better understanding of the effect of N status of the plant on the efficiency of N supplied trough the leaves is important to increase efficacy of foliar N recommendation in commercial sugarcane fields.

A persistent nutrient deficiency may impact crop phenology, reducing canopy growth, change the morphological and chemical structure of leaves, and reduce absorption. In opposite, short-term deficiencies can increase absorption by increasing the activity of deficiency response mechanisms (“absorption activators”), or as a consequence of the relative abundance of unsaturated sites linked with the deficient nutrients (Fernández, Sotiropoulos and Brown, 2015).

Nitrogen deficiency in citrus leaves increased the concentration of epicuticular wax (Bondada et al., 2006; Bondada et al., 2001). A similar response was seen in *Pinus palustris* with low N content, which had higher concentrations of epicuticular wax than those with high N content (Prior et al., 1997). The rise in epicuticular wax alters the morphology of the epicuticular wax by decreasing foliar absorption, decreasing the trans-cuticular transport mechanism, and raising the fraction of long-chain alkanes (Chiu et al., 1992). A lack of N can also have an impact on uptake, which can reduce leaf expansion and shoot growth, leading to smaller leaves and stems with thicker cuticles and more epicuticular wax per unit leaf area.

However, the impact of the N status of the plant on the capacity of leaves to absorb leaf-applied N is still a matter of controversy. Nitrogen was more readily absorbed through the leaves of olive trees lacking in N than in plants with optimal content (Fernandez-Escobar et al., 2011). In opposite, citrus foliar absorption of N from urea decreased as the N foliar content increased (Leacox; Syvertsen, 1995). Absorption and translocation of foliar urea-N in the fall was more effective in *Hydrangea* and apple plants with low N status than in those with higher N status, which was accounted for by high N feedback inhibition (Cheng et al. 2002; Bi and Scagel 2008).

Since foliar application of urea-N is becoming a common practice in sugarcane cultivation, replacing part of N applied to the soil or simply complementing N fertilization, it is imperative to understand the impact of sugarcane N nutrition on the uptake and translocation of foliar applied N. Our objective here was to determine the best N ratio to growth sugarcane under nutrient solution and its impact on the efficiency of N uptake by sugarcane leaves.

1.2 Material and Methods

1.2.1 Cultivation of plants

The experiment was conducted in a growth chamber at the Department of Soil Science at ESALQ/USP. The growth chamber was maintained at constant temperature of 32 $\pm$ 2 °C during daylight hours and 18 $\pm$ 2 °C at nighttime throughout the duration of the experiment, which had a 12-hour photoperiod. Throughout cultivation, the relative humidity in the growth chamber was kept at 80%.

Sugarcane seedlings were obtained from two-centimeter-long stalks pieces (containing one bud per stalk piece) of a nine months old plant. The RB966928 variety was chosen because its representative sugarcane cultivation area in Central South Brazil. The stalk pieces with one bud were then treated with fungicide and put in plastic trays with washed sand as the substrate (Figure 1A, supplementary). The seedlings were irrigated daily with deionized water and, twenty-one days after planting, thirty seedlings of uniform size were chosen to be transplanted into the nutrient solution pots.

In the nutrient solution stage, pots with 3 L volume capacity were chosen. Sugarcane seedlings were transplanted and were kept suspended by a foam tightly maintained in the orifices of the lids, to prevent it to fall (Figure 1B, supplementary). For cultivation, Hoagland and Arnon's solution (1950) was used, containing 7.5 mM NH_4NO_3 (100%), 1 mM KH_2PO_4, 2 mM $MgSO_4$, 5 mM $CaCl_2$, 5 mM KCl, 3.5 mM K_2SO_4. Micronutrients were supplied as boron H_3BO_3 (0.5 µM), copper $CuSO_4.5H_2O$ (0.02 µM), iron Fe EDTA (5 µM), manganese $MnSO_4.H_2O$ (0,5 µM) and zinc $ZnSO_4$ (0.05 µM).

When transplanting the seedlings, the nutrient solution was prepared with only 50% of the ionic strength, so that the plants could adapt to the new environment. In the first change of solution, which occurred seven days after transplanting, the nutrient solution was prepared with 100% of ionic strength. The solution was changed every seven days, four changes were performed throughout the experiment. The pots and lids were painted with silver spray to avoid the increase in temperature of the nutrient solution and the bench received Styrofoam plates to avoid excess heat. Daily, the pH of the solution was adjusted to 5.8 using 0.5 M HCl or 0.5 M NaOH.

1.2.2 Experimental design

The experimental design was a randomized blocks in a factorial 5x2, as follows: 5 supplies of N (as NH_4NO_3), 0.75, 1.5, 3.0, 7.5 and 15 mM of N (representing 5, 10, 20, 50 and 100% of the N supply of the Hoagland and Arnon (1950) original solution), and 2 supplies of N through the leaves: control (without foliar spray) and treated (with 0.15 g N $plant^{-1}$ as urea). Before receiving the foliar N spray, the plants were grown for a period of 30 days and presented approximately 60 cm.

The application of N-urea was performed using 1.5 ml of a solution with 0.1% tween 20 (surfactant) and 10% of N, totalizing 150 mg $plant^{-1}$. This N rate was based on application of 10 kg ha^{-1} N, considering a tillering of 10 stalks m^{-1}. A brush was used to apply the solution on the abaxial and adaxial faces of the 3 youngest leaves of the plant. The leaves that received the application were marked with a string for identification (Figure 1).

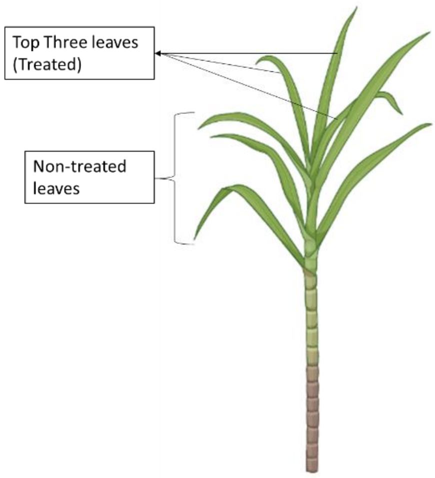


Figure 2. Scheme of foliar urea-N application to the plants cultivated in nutrient solution.

1.2.3 Measurements

After 10 days of foliar spray application, the experiment ended with the collection of plant compartments. Treated leaves, untreated leaves, stems, and roots were separated. The leaves were washed in the following sequence to remove urea-solution adhered to the leaf surface: distilled water with 1 ml l^{-1} of neutral detergent, solution with 0.1 M of HCl and distilled water. All the plant compartments were weighed before and after drying in a oven at

50ºC for 72 hours, being ground in a Wiley-type mill. The determination of N, P, K, Ca, Mg, S content in the samples was performed according to the methodology described by Malavolta, Vitti and Oliveira (1997).

1.2.4 Statistical analysis

The Shapiro-Wilk and Levene tests (P>0.1) were used to assess the assumptions of normality and variance homogeneity. When Grubbs' test detected outliers, they were removed. The F-test was used to submit data to ANOVA. When the F-test was significant (P<0.1), a regression test was used to model the effect of N rate (linear, quadratic, and asymptotic) on plant parameters. The LSD test was used to differentiate the effect of foliar N spray (P<0.1)

1.3 Results

The supply of N in the solution had a direct effect on plant growth and biomass accumulation. Hoagland and Arnon's solution (1950) at its total N concentration (15 mM, 100%) did not generate the highest biomass production. Highest biomass production of all plant compartments was obtained by the 3.0 mM of N solution (20%). The rates of 0.75 (5%) and 1.5 mM of N (10%) caused N deficiency in plants. In the same direction, lower dry mass of plants was obtained by a supply of 50% and 100% N in the nutrient solution. There was no linear model adjustment in dry mass production, and the treatment with 20% of the N supply achieved 33% more than the 50 and 100% supplies (Figure 2, a). Plants fed N-deficient nutrition (0.75 mM) spent 61% of their biomass on root formation, whereas plants fed adequate N (15 mM) allocated 35% of their biomass on roots. Plants with higher development, i.e., 20% N supply, allocated 43% of biomass to roots, 26% to stem, and 30% to leaves. (Figure 2, b)

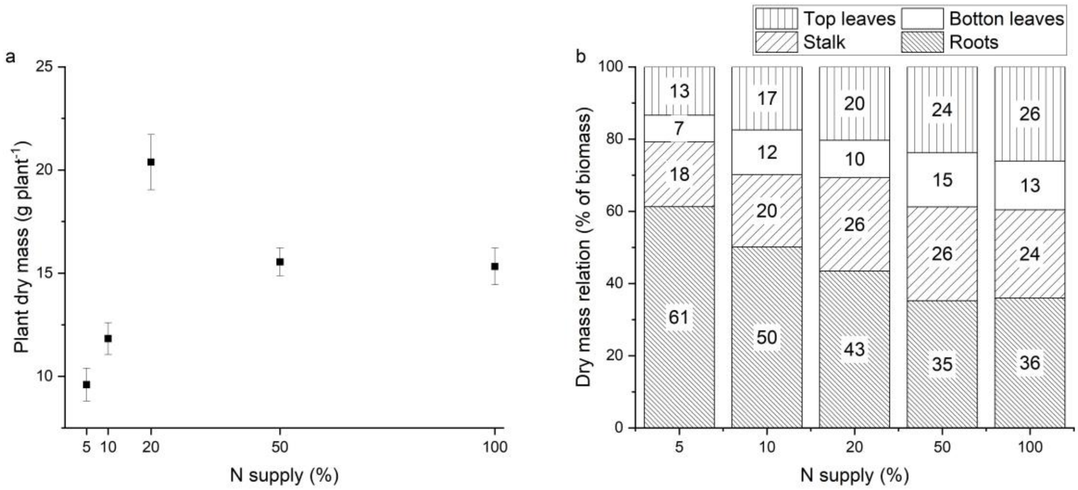


Figure 2. a) Averages of dry mass under the influence of N supplies in the nutrient solution (there was no adjustment of linear models), b) Dry mass relation in each N supply treatment.

The foliar N application caused a decrease in dry mass production in top leaves (-10%), stems (-18%), and roots (-18). No effect on bottom leaves was observed. On average, plants that received foliar N spray had a 15% reduction in dry mass (Figure 3), which can be attributed to a toxic effect of the N rate adopted in foliar spray.

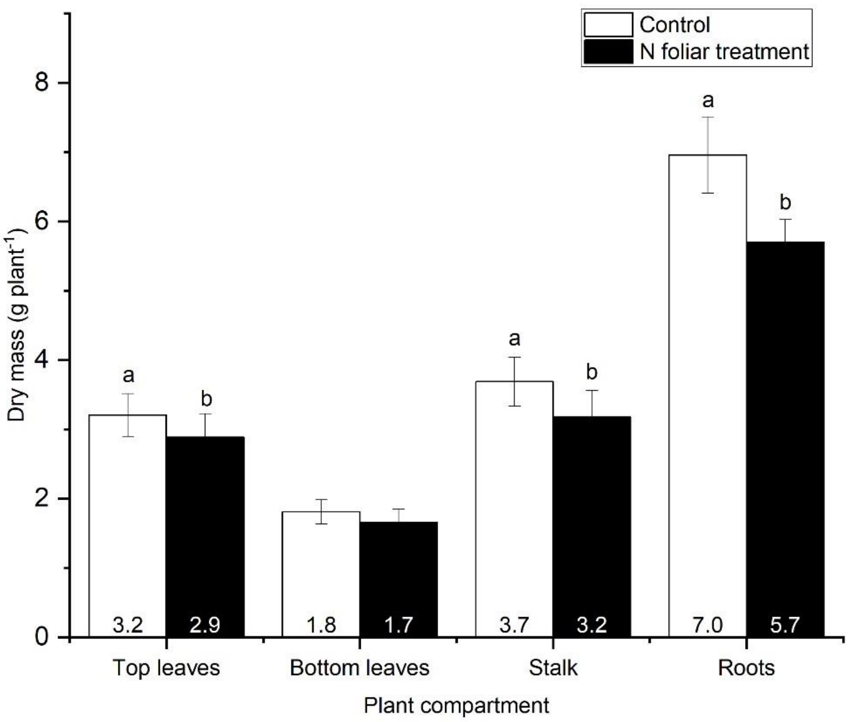


Figure 3. Average dry mass under the influence of N foliar application. LSD test was performed to evaluate mass in each plant compartment (P<0.1).

The N supply in the nutrient solution influenced the biomass production of all plant compartments, and also influenced the uptake of the other nutrients (N, P, K, Ca, Mg, S). Nitrogen uptake increased linearly with N supply and N supply above 20%, generated a decrease in the uptakes of K, Ca, Mg and S (Figure 5, Table S1).

The root: shoot ratio, i. e. root dry mass ratio divided by shoot dry mass, was affected by N supply. The lowest N supply (5%) obtained 0.65 g plant^{-1} of shoot dry mass for each gram of root, ratio of 0.65:1. The highest N supply (100%) generated a root: shoot ratio of 1:1.8, that is, 2.8 times higher compared to plants supplied with 5% N (Figure 4.a). The N concentration in the plant, i.e., total uptake divided by the total dry mass, increased linearly with increased N supply. While the 5% N supply provided 0.87% N, the treatment with 100% N generated a final concentration of 3.14% N, representing a 3.6 times higher N concentration (Figure 4.b). The plants with the best N content in the nutrient solution, that is, supplied with 20% N, had a shoot: root ratio of 1:1.36 and 1.3% of N concentration (Figure 4.a).

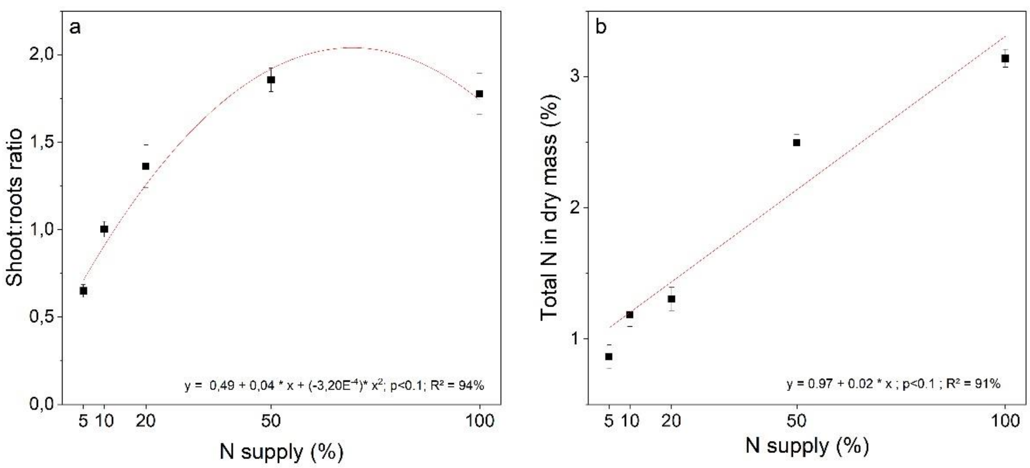


Figure 4. Representation of shoot: root dry mass ratio (a) and total N content (b) according to the percentage of N in the N solution. Models were fitted with analysis of variance at $p<0.1$.

The accumulation of N, P, K, Ca, Mg, and S were influenced by the supply of N in the nutrient solution. The N uptake was linearly increased by the supply of N, independently of the reduction in biomass production caused by N rates of 50 and 100% in the nutrient solution. The other nutrients did not reveal a linear increase according to the N rate in the nutrient solution. Phosphorus had an asymptotic uptake curve, thus the demand for P did not follow the highest with biomass accumulation. The nutrients K, Ca, Mg, and S were unable to fit a significant linear model, however, the values were evaluated using the LSD mean test (p<0.1). The uptake of these nutrients follows the dry mass production, that is, the supplies of 5 and 10% were the lowest uptakes, the rate of 50 and 100 intermediate and the rate of 20% N represented the highest uptake (Figure 5). Those results indicates that N accumulation presented a different behavior compared to the other nutrients, indicating a possible toxic effect of the highest N rates in the nutrient solution.

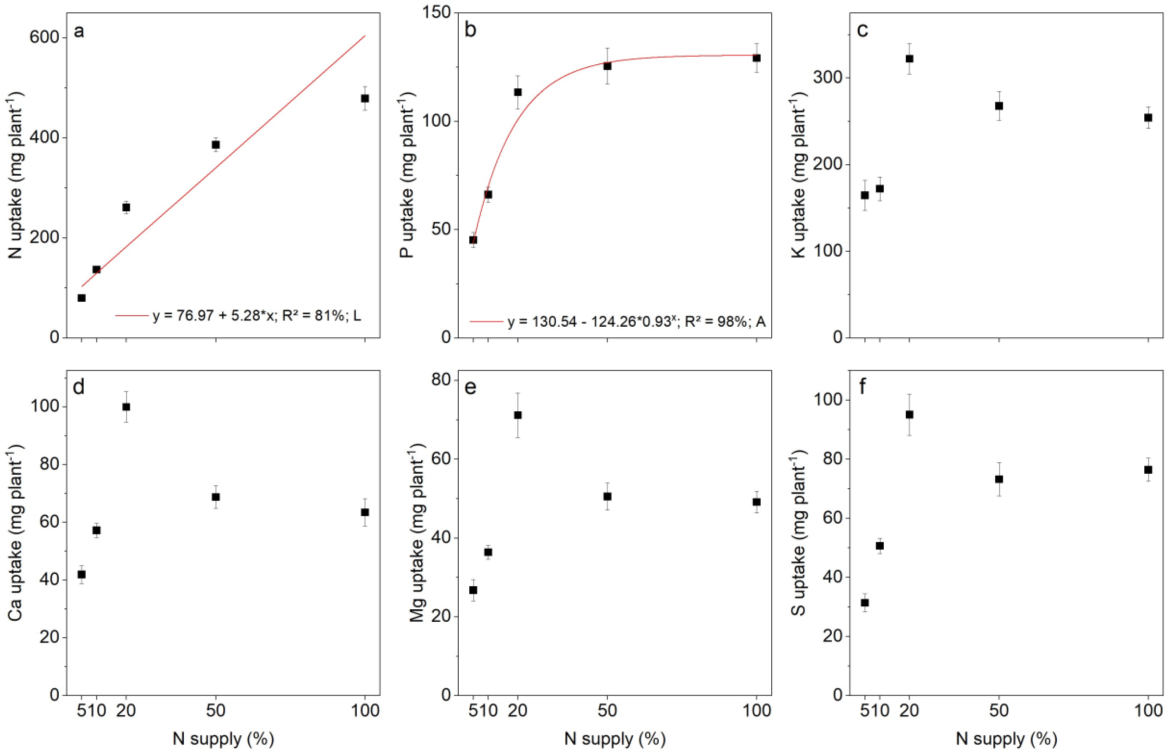


Figure 5. Uptake (accumulation) of N, P, K, Ca, Mg and S in all plant compartments (on the average of control + N foliar spray treatments). a) total N uptake (fitted as linear model), b) total P uptake (fitted as asymptotic model), c) total K uptake (no linear model fitted), d) total Ca uptake (no linear model fitted), e) total Mg uptake (no linear model fitted), and f) total S uptake no linear model fitted).

The top leaves did not have the P, Mg, and S contents influenced by the supply of N in the nutrient solution (Figure 5). Among plant compartments, only the content of N in top leaves suffered interaction with the application of foliar N (Figure 6). The leaves of plants with low N supply (5 and 10%) had the highest N content increments. On average, the N content increased by 55% in the treated leaves, and the levels increased by 129 and 84% respectively for the 5 and 10% N supplies. The 20% N supply had lower N content in the treated plants and not treated.

The bottom leaves were influenced by the supply of N in all mineral contents and uptakes. The bottom leaves plants treated with foliar N did not show changes in nutrient contents, except for N, which increased by an average of 15%. There was an interaction between the application of N and the Ca content, and the supply of 5% of N had Ca contents 25% higher compared to the untreated ones (Table S3).

Root N concentrations increased with N supply and foliar N application. Nitrogen contents increased with N supply in all compartments (treated and non-treated leaves, stalks, and roots). As shown in Supplementary Tables 2, 3, 4, and 5, the N contents increased in all compartments, but there was interaction in the applied leaves, with the leaves with the lowest N supply having the highest concentration increment (Figure 6).

The plants treated with N foliar and supplied with 5 and 10% had N content increments of 129 and 82%, respectively, compared to the control plants. The plant supplied with 20% of N, had the highest N uptake increase in the treated leaves. The top leaves of control plants, 20% of N supplied, had an uptake of 42.6 mg plant^{-1} while the treated ones 97.5 mg plant^{-1}, an increase of 128% (Figure 6, Table S2).

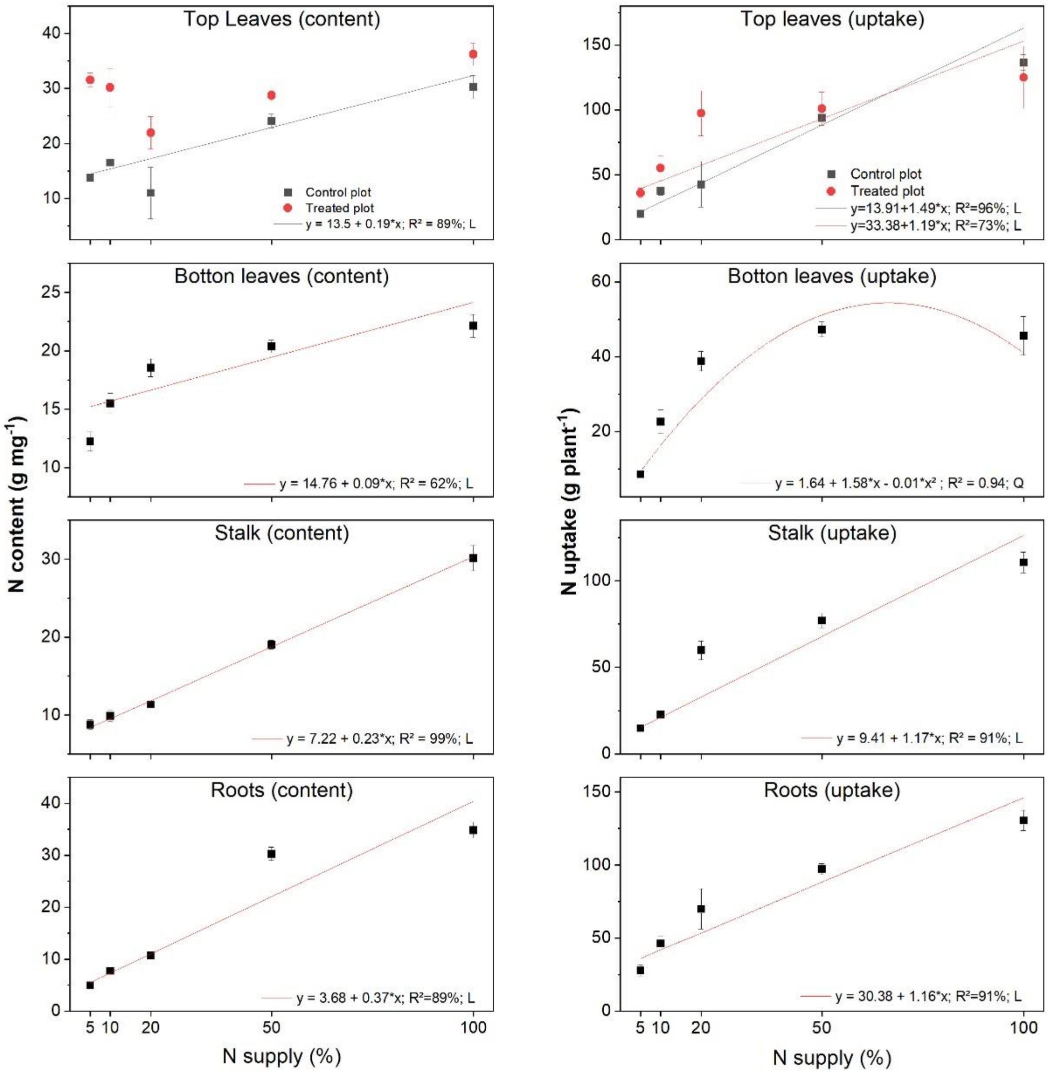


Figure 6. N content and uptake in treated leaves (top leaves), untreated leaves (bottom leaves), stalk and roots. The treated leaves or top leaves presented interaction between N supply and N foliar application ($P<0.1$), so they were presented separately. Untreated leaves, stalk and roots presented no interaction ($P>0.1$), so the curve was presented as averaged.

1.4 Discussion

The Hoagland and Arnon nutrient solution have been widely used in hydroponic systems and is considered a standard reference for plant nutrient requirements (HOAGLAND, ARNON, 1950). In our work, it was found that the concentration of 15 mM of N, as suggested by the authors, is excessive, at least during the initial growth of the plants, since the optimal supply for plant development was only 20% of the total N concentration (3 mM). The highest rates presented negative effect on growth for early development stages, and the high concentration of N mostly inhibited root growth (Figure Supplementary A)

Excessive nitrogen (N) in the Hoagland and Arnon (1950) nutrient solution can have negative effects on initial sugarcane growth, as presented in this study. High levels of N can result in excessive vegetative growth, which can delay or reduce the development of the root system, leading to reduced water and nutrient uptake. Franco et al. (2015), in a field study, found that excessive N application reduced sugarcane root growth and increased above-ground biomass, generating negative consequences for economic profitability and the environment.

Otto et al. (2009) investigated methods for measuring sugarcane roots in field trials and discovered that N fertilizer at planting generated changes in the distribution of the root system in the soil rather than not promoting increased root accumulation in the cane plant. The distribution of the root system in the soil profile was improved by the absence of N fertilizing. Thus, the results of Otto et al. 2009 were aligned to the findings of the nutrient solution experiment presented herein, in which excess of N fertilization reduced root growth, generating an imbalance in the shoot: root ratio.

Although the plants with the highest N supply had a smaller dry mass than the plants with a 20% N supply, the total N uptake increased linearly with the increase in the N supply (Figure 4). This indicates a luxury consumption, the excess of N supply resulted in an accumulation of nutrients in the plant tissues. Potassium (K) concentration in leaves raised with increasing N supply, but the concentration in roots and stems was maintained.

Plants can sense K^+/ NO_3^- levels in soils and adjust accordingly the uptake and root-to-shoot transport to balance the distribution of these ions between tissues (Raddatz et al. 2020). At the destination, NO_3^- is stored inside vacuoles using K^+ as counterion, where both ions contribute to osmotic adjustment (Barragan et al., 2012; Martinoia et al., 2012). Symptoms of K deficiency were observed in the leaves of plants supplied with 50 and 100% N. The higher concentration of K in the leaves supplied with more N was not enough for the plants not to show symptoms of K deficiency, that is, there was probably an osmotic imbalance due to the high nitrate content in the leaves.

Calcium concentration in leaves had a negative relationship with N content (Table S2 and S3). Calcium is a cation responsible for controlling NH_4 uptake (Saito and Uozumi, 2020). As the NH_4^+ uptake must be tightly managed because of toxicity of NH_4+ the influx of the compound is regulated by two transporters (AMT1;2, AMT1;2). These transporters are activated by elevation of intracellular Ca to increase NH_4^+ influx (Liu et al. 2017).

The foliar application was calculated considering a rate of 10 kg ha^{-1} N, which is often used in commercial sugarcane fields. Some toxicity effects were observed in leaves that received N foliar spray application. Some chlorotic bands were noted on the treated leaves (Figure Supplementary B). The foliar N rate used in this work may have exceeded the plant tolerance, especially for the early development stage evaluated in this study. In addition to the toxicity of free ammonia, an ion that needs to be quickly incorporated in glutamine (Witte, 2011), urea needs to be hydrolyzed in the leaf, which can also be very dangerous if the pH of the cytosol rises (Bremner, 1995; Krogmeier et al., 1989). The effects of an elevation in cytosolic pH on protein structure and function, ion transport, and signaling pathways can be detrimental to cellular survival and function.

One of the important functions which can be affected by the pH increase is the dissociation of abscisic acid hydroxyl (ABAH) into ABA-abscisate, which is a role in regulating stomatal closure compound. When there is a water deficit, ABA- dissociates for transport to guard cells because the higher cell pH drives ABA synthesis, which in turn initiates stomatal closure (Taiz et al., 2017). ABA has been shown to promote root growth and inhibit shoot growth, and it has been suggested to play a role in regulating plant architecture and branching patterns.

The absorption of foliar spray N applied can be confirmed by the higher total N uptake in the treated plant (Table S1). Even with lower dry mass, possibly caused by the toxicity of the N foliar supply, the treated plants had a higher N uptake. The highest difference between uptake was in the plant supplied with 20% of N, the uptake was 35.1 mg plant^{-1} higher, even with the plant with 13% less dry mass (Table S1). The plant with a 20% N supply was the only one that had the leaf dry mass increased with the application of foliar N, which indicates that the nutritional balance is extremely important for the decision to N foliar apply.

1.5 Conclusion

A decision maker has to consider a number of aspects before applying foliar fertilizer. This study focused on the plant nutrient status, which includes nutrient availability and crop growth stage. Nutrient solution with 20% of the recommended rate of the Hoagland and Arnon (1950) solution (3 mM of N) promoted highest biomass production and N accumulation of young sugarcane plants, being indicated for sugarcane cultivation under nutrient solution.

Plants with excess N in the nutrient solution have an impaired root: shoot ratio, by reducing the root growth caused by the excessive N supply. The plants with the highest development (20% N in the nutritive solution) obtained a root/shoot ratio of 1:1.3, while the N-deficient 1:1.6 and plants with N-excess, 1:0.5. This finding might help to explain why sugarcane may not respond to high N rates under field cultivation. The excessive amount of N supplied at the beginning of the growth stage may have an impact on the root: shoot ratio, resulting in a plant with a larger shoot than the root system can support at that stage of growth.

References

BARRAGÁN, V.; LEIDI, E. O.; ANDRÉS, Z.; RUBIO, L.; DE LUCA, A.; FERNÁNDEZ J. A. Ion exchangers NHX1 and NHX2 mediate active potassium uptake into vacuoles to regulate cell turgor and stomatal function in *Arabidopsis*. **Plant Cell,** v. 24, p. 1127–1142, 2012.

BI, G.; SCAGEL, C. F. Nitrogen uptake and mobilization by Hydrangea leaves from foliar-sprayed urea in fall depend on plant nitrogen status. **Horticultural Science**, v. 43, p. 2151–2154, 2008.

BONDADA, B. R.; PETRACEK, P. D.; SYVERTSEN, J. P.; ALBRIGO, L. G. Cuticular penetration characteristics of urea in citrus leaves. Journal of **Horticultural Science & Biotechnology**, v. 81, p. 219-224, 2006.

BONDADA, B. R.; SYVERTSEN J. P.; ALBRIGO, L. G. Urea nitrogen uptake by citrus leaves. **Horticultural Science**, v. 36, p. 1061–1065, 2001.

BREMNER, J. M. Recent research on problems in the use of urea as a nitrogen fertilizer. **Fertilizer Research**, v. 42, p 321-329, 1995

BREMNER, J. M.; KROGMEIER, M. J. Evidence that the adverse effect of urea fertilizer on seed germination in soil is due to ammonia formed through hydrolysis of urea by soil urease. **Proceedings of the National Academy of Sciences**, v. 86, p. 8185-8188, 1989.

CANTARELLA, H.; TRIVELIN. P. C. O.; VITTI. A. C.; Nitrogênio e enxofre na cultura da cana-de-açúcar. In: YAMADA. T.; ABDALLA. S. R. S.; VITTI, G. C. **Nitrogênio e enxofre na agricultura brasileira.** Piracicaba: IPNI Brasil. 2007. p. 407-464.

CHENG, L.; DONG, S.; FUCHIGAMI, L. H. Urea uptake and nitrogen mobilization by apple leaves in relation to tree nitrogen status in autumn. **Journal of Horticultural Science & Biotechnology**, v. 77, p. 13-18, 2002.

CHIU, S. T.; ANTON, L. H.; EWERS, F. W.; HAMMERSCHMIDT, R.; PREGITZER, K. S. Effects of fertilization on epicuticular wax morphology of needle leaves of douglas-fir, *Pseudotsuga menziesii* (pinaceae). **American Journal of Botany**, v. 79, p. 149-154, 1992.

FAGERIA, N. K.; BARBOSA FILHO, M. P.; MOREIRA, A.; GUIMARÂES, C. M. Foliar fertilization of crop plants. **Journal of Plant Nutrition**, v. 23, p. 1044-1064, 2009.

FERNÁNDEZ, V.; SOTIROPOULOS, T.; BROWN, P. (2015) **Adubação foliar: Fundamentos Científicos e Técnicas de Campo**. Abisolo, São Paulo, 150 p.

FERNANDEZ-ESCOBAR, R.; GARCIA-NOVELO, J. M.; RESTREPO-DIAZ, H. Mobilization of nitrogen in the olive bearing shoots after foliar application of urea. **Scientia Horticulturae**, v. 127, p. 452-454, 2011.

FRANCO, H. C. J. F.; OTTO, R.; VITTI, A. C.; FARONI, C. E.; OLIVEIRA, E. C. A.; FORTES, C.; FERREIRA, D. A.; KOLLN, O. T.; GARSIDE, A. L.; TRIVELIN, P. C. O. Residual recovery and yield performance of nitrogen fertilizer applied at sugarcane planting. **Scientia Agricola**, v. 72, p. 526-534, 2015.

FRANCO, H.C.J.; OTTO, R.; FARONI, C.E.; VITTI, A.C.; OLIVEIRA, E.C.A.; TRIVELIN, P.C.O. Nitrogen in sugarcane derived from fertilizer under Brazilian field conditions. **Field Crops Research**, Amsterdam, v. 28, p. 29-41, 2011.

GUELFI, D. Fertilizantes nitrogenados estabilizados, de liberação lenta ou controlada. **Informações Agronômicas**, v. 157, p. 1-14, 2017.

HOAGLAND, D.; ARNON, D. I. **The water culture method for growing plants without soil.** California Agriculture Experimental Station, 1950, v. 347, 33 p.

KROGMEIER, M. J.; MCCARTY, G. W.; BREMNER, J. M. Phytotoxicity of foliar-applied urea. **Proceedings of the National Academy of Sciences**, v. 86, p. 8189–8191, 1989

LEACOX, J. D.; SYVERTSEN, J. P. Nitrogen uptake by citrus leaves. **Journal of the American Society for Horticultural Science**, v. 120, p. 505-509, 1995.

LIU, K. H.; NIU, Y.; KONISHI, M.; WU, Y.; DU, H.; SUN CHUNG, H.; LI, L.; BOUDSOCQ, M.; MCCORMACK, M.; MAEKAWA S, ISHIDA T, ZHANG C, SHOKAT K, YANAGISAWA S, SHEEN J. Discovery of nitrate-CPK-NLP signaling in central nutrient-growth networks. **Nature**, v. 18, p v. 545, p. 311-316, 2017.

MALAVOLTA, E.; VITTI, G. C.; OLIVEIRA, S. A. **Avaliação do estado nutricional das plantas**: princípios e aplicações. 2.ed. Piracicaba: Associação Brasileira para Pesquisa da Potassa e do Fosfato, 1997. 319 p.
MARTINOIA E.; MEYER S.; DE ANGELI A.; NAGY R. Vacuolar transporters in their physiological context. **Annual Review of Plant Biology**, *v.* 63, p. 183–213, 2012.

MEYER, J.; SCHMANN, A.; WOOD, R.; NIXON, D.; VAN DEN BERG, M. Recent advances to nitrogen use efficiency of sugarcane in the South African sugar industry. **Proceeding International Society Sugar Cane Technology,** v, 26, p. 238-246, 2007.

OTTO, R.; TRIVELIN, P. C. O.; FRANCO, H. C. J.; FARONI, C. E.; VITTI, A. C Root system distribution of sugar cane as related to nitrogen fertilization, evaluated by two methods: monolith and probes. **Revista Brasileira de Ciência do Solo**, v. 33, p. 3, 2009.

PRIOR, S. A.; PRITCHARD, S. G.; RUNION, G. B.; ROGERS, H. H.; MITCHELL, R. J. Influence of atmospheric CO2 enrichment, soil N, and water stress on needle surface wax formation in *Pinus palustris* (pinaceae). **American Journal of Botany**, v. 84, p. 1070- 1077, 1997.

RADDATZ, N.; MORALES DE LOS RÍOS, L.; LINDAHL, M.; QUINTERO, F. J.; PARDO, J. M. Coordinated Transport of Nitrate, Potassium, and Sodium. **Frontiers in Plant Science**, v. 6, p. 11:247, 2020.

SAITO, S.; UOZUMI, N. Calcium-Regulated Phosphorylation Systems Controlling Uptake and Balance of Plant Nutrients. **Frontiers in Plant Science**, v. 11, p 11-44, 2020.

TAIZ, L.; ZEIGER, E.; MOLLER, I.; MURPHY, A. **Fisiologia e desenvolvimento vegetal**. 6.ed. Porto Alegre: Artmed, 2017. 888 p.

VILLALBA, H. A. G.; LEITE, J. M.; OTTO, R.; TRIVELIN, P. C. O. Fertilizantes nitrogenados: novas tecnologias. **Informações Agronômicas**, v. 148, p. 12-18, 2014.

WITTE, C. P. Urea metabolism in plants. **Plant Science**, v. 180, p. 431-438, 2011.

Supplementary material

Table S1. Dry mass of treated leaves, non-treated leaves, stalks, roots, and total dry mass; and the N, P, K, Ca, Mg and total uptake by the plant.

N supply	N foliar	Treated leaves	Non-treated leaves	Stalk	Roots	Total dry mass	N	P	K	Ca	Mg	S
%	-	(dry mass) g plant^{-1}					(uptake) mg plant^{-1}					
5	No	1,4	0,8	1,9	6,9	11,1	74,0	50,6	197,5	44,8	31,1	35,8
10	No	2,3	1,6	2,8	6,7	13,4	131,8	72,0	182,3	62,0	39,4	53,0
20	No	3,9	2,3	5,5	9,9	21,6	243,9	115,8	333,1	101,0	72,5	98,2
50	No	3,9	2,4	4,1	5,8	16,3	382,2	136,8	280,8	72,1	54,1	79,7
100	No	4,5 a	2,0	4,1	5,4	16,0	481,2	128,9	261,1	63,9	50,0	76,3
5	Yes	1,1	0,6	1,5	4,8	8,1	86,4	40,0	131,9	39,1	22,4	27,1
10	Yes	1,9	1,4	1,9	5,2	10,3	142,2	60,4	162,2	52,4	33,4	48,5
20	Yes	4,4	1,9	4,7	7,8	18,8	278,9	111,1	311,2	98,8	69,9	92,1
50	Yes	3,5	2,3	3,9	5,1	14,8	390,8	114,2	254,9	65,4	46,9	66,8
100	Yes	3,5 b	2,2	3,4	5,6	14,7	477,2	129,6	247,4	63,0	48,3	76,7
5		1,3	0,7	1,7	5,9	9,6 d	80,2	45,3	164,7	41,9	26,8	31,5
10		2,1	1,5	2,4	5,9	11,8 c	137,0	66,2	172,2	57,2	36,4	50,7
20		4,2	2,1	5,1	8,9	20,2 a	261,4	113,4	322,2	99,9	71,2	95,1
50		3,7	2,3	4,0	5,5	15,5 b	386,5	125,5	267,8	68,7	50,5	73,3
100		4,0	2,1	3,7	5,5	15,3 b	479,2	129,2	254,3	63,4	49,1	76,5
Control		3,2 A	1,8	3,7 A	7,0 A	15,7	262,6	100,8	251,0	68,8	49,4	68,6
Treated		2,9 B	1,7	3,1 B	5,7 B	13,4	275,1	91,1	221,5	63,7	44,2	62,2
p N supply		<0,01*	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*
p N foliar		0,06*	0,33	0,06*	0,01*	<0,01*	0,35	0,11	0,05*	0,22	0,14	0,19
p N supply x N spray		0,07*	0,76	0,92	0,43	0,91	0,91	0,77	0,79	0,96	0,96	0,91

N supply was evaluated by the regression (p<.1); N spray was evaluated by the LSD test (p<.1). Different letters mean rates differences.

Table S2. Top leaves (treated) mineral contents and uptakes (N, P, K, Ca, Mg and S) by the plants under the influence of N supplies and foliar N application.

N supply	N foliar	N	P	K	Ca	Mg	S	N	P	K	Ca	Mg	S
%	**-**	**---------------------------- (content) g kg^{-1}**					**----------------------------**	**----------------------------(uptake) mg plant^{-1}**					**----------------------------**
5	No	13,8 B	6,3	12,1	5,0 B	1,8	1,8	19,8	9,0	17,4	7,1	2,6	2,6
10	No	16,5 B	5,7	8,7 B	5,0 B	1,7	1,9 B	37,5	12,9	19,9	11,4	3,9	4,2
20	No	11,0 B	5,3	12,1	4,0 B	1,6	2,0	42,6 B	20,9	47,2	15,7	6,3	7,8
50	No	24,1 B	5,7	15,8	4,2	1,7	2,0	93,7	22,2	61,3	16,4	6,5	7,9
100	No	30,2 B	6,1	15,4	3,9	1,6	2,0	136,3	27,5	69,4 A	17,8	7,2	9,2
5	Yes	31,6 A	7,0	10,3	9,0 A	2,2	2,1	35,8	7,9	11,8	10,3	2,5	2,4
10	Yes	30,1 A	6,7	12,8 A	7,2 A	2,1	2,5 A	55,1	12,5	23,4	13,0	3,8	4,5
20	Yes	21,9 A	5,5	13,0	5,2 A	2,0	2,0	97,5 A	24,3	57,5	22,8	8,8	8,8
50	Yes	28,7 A	5,5	16,0	4,1	1,9	2,1	100,8	19,7	56,7	14,7	6,7	7,3
100	Yes	36,2 A	6,6	14,2	4,5	1,9	1,9	124,9	23,4	49,6 B	15,6	6,9	6,6
5		22,7	6,6	11,2	7,0	2,0	2,0	27,8	8,5	14,6	8,7	2,6	2,5
10		23,3	6,2	10,7	6,1	1,9	2,2	46,3	12,7	21,6	12,2	3,8	4,4
20		16,5	5,4	12,5	4,6	1,8	2,0	70,0	22,6	52,3	19,3	7,6	8,3
50		26,4	5,6	15,9	4,2	1,8	2,0	97,3	21,0	59,0	15,6	6,6	7,6
100		33,2	6,4	14,8	4,2	1,8	2,0	130,6	25,5	59,5	16,7	7,1	7,9
Control		19,1	5,8	12,8	4,4	1,7 B	1,9	66,0	18,5	43,0	13,7	5,3	6,3
N foliar		29,7	6,3	13,3	6,0	2,0 A	2,1	82,8	17,6	39,8	15,3	5,8	5,9
p N supply		<0,01	0,27	<0,01	<0,01*	0,63	0,62	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*
p N foliar		<0,01	0,29	0,48	<0,01*	0,01*	0,08	<0,01*	0,59	0,31	0,19	0,43	0,39
p N supply x N spray		<0,01	0,91	0,05*	<0,01*	0,98	0,09	<0,01*	0,73	0,07*	0,11	0,48	0,18

N supply was evaluated by the regression (p<.1); N spray was evaluated by the LSD test (p<.1). Different letters mean rates differences

Table S3. Bottom leaves (non-treated) mineral contents and uptakes (N, P, K, Ca, Mg and S) by the plants under the influence of N supplies and foliar N application.

N supply	N foliar	N	P	K	Ca	Mg	S	N	P	K	Ca	Mg	S
%	-	------------ (content) g kg^{-1} ------------						------------ (uptake) mg plant^{-1} ------------					
5	No	10,7	8,0	10,5	12,0 B	4,0	2,2	8,4	6,3	8,2	9,4	3,1	1,7
10	No	13,7	8,5	12,4	13,0	3,7	3,0	21,6	13,2	19,4	20,5	5,8	4,7
20	No	17,5	7,4	13,6	12,9	3,7	3,5	40,5	17,0	31,4	29,9	8,7	8,2
50	No	19,8	13,8	16,1	11,2	3,7	2,7	47,2	33,1	38,7	26,8	8,7	6,5
100	No	21,0	13,4	14,0	9,9	3,2	2,6	41,2	26,6	27,3	19,6	6,3	5,1
5	Yes	13,7	9,2	10,4	15,1 A	4,6	2,7	8,7	5,8	6,5	9,5	2,9	1,7
10	Yes	17,3	8,0	14,3	11,2	3,3	3,3	23,6	10,6	20,4	14,4	4,3	4,2
20	Yes	19,6	8,0	12,8	14,5	4,5	3,8	37,1	15,0	24,0	26,9	8,3	7,1
50	Yes	21,0	11,2	11,9	9,8	3,0	2,2	47,4	25,5	27,1	22,4	6,8	5,1
100	Yes	23,3	13,5	14,0	9,8	3,3	2,5	49,9	29,3	31,2	21,7	7,5	5,3
5		12,2	8,6	10,4	13,6	4,3	2,4	8,6	6,1	7,4	9,5	3,0	1,7
10		15,5	8,2	13,3	12,1	3,5	3,1	22,6	11,9	19,9	17,5	5,1	4,4
20		18,6	7,7	13,2	13,7	4,1	3,7	38,8	16,0	27,7	28,4	8,5	7,7
50		20,4	12,5	14,0	10,5	3,3	2,5	47,3	29,3	32,9	24,6	7,7	5,8
100		22,1	13,4	14,0	9,9	3,3	2,5	45,6	28,0	29,3	20,7	6,9	5,2
Control		16,5 B	10,2	13,3	11,8	3,7	2,8	31,8	19,3	25,0	21,2	6,5	5,2
N foliar		19,0 A	10,0	12,7	12,1	3,7	2,9	33,4	17,3	21,9	19,0	6,0	4,7
p N supply		<0,01*	<0,01*	0,02*	<0,01*	0,02*	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*
p N foliar		<0,01*	0,63	0,37	0,64	0,76	0,57	0,58	0,36	0,27	0,2	0,4	0,2
p N supply x N spray		0,76	0,19	0,12	0,06*	0,22	0,27	0,74	0,65	0,43	0,59	0,59	0,71

N supply was evaluated by the regression (p<.1); N spray was evaluated by the LSD test (p<.1). Different letters mean rates differences.

Table S4. Stalk mineral contents and uptakes (N, P, K, Ca, Mg and S) by the plants under the influence of N supplies and foliar N application.

N supply	N spray	N	P	K	Ca	Mg	S	N	P	K	Ca	Mg	S
%	-	(stalk) g kg^{-1}						(stalk) g plant^{-1}					
5	No	7,5	4,8	18,5 B	2,9	2,3	2,6	14,3	9,2	35,5	5,6	4,3	5,0
10	No	8,5	4,5	17,1 B	2,5	2,0	2,5	23,7	12,4	48,1	7,0	5,7	7,0
20	No	11,2	4,2	19,7	2,4	1,8	2,5	61,1	22,9	108,1	12,8	9,6	13,3
50	No	19,0	5,0	19,1 B	2,0	2,0	2,3	79,1	20,7	78,7	8,4	8,3	9,4
100	No	28,5	6,8	20,0	2,4	2,1	3,4	113,9	27,4	80,8	9,5	8,7	13,2
5	Yes	10,1	6,5	21,2 A	4,1	2,6	4,0	15,5	9,9	32,6	6,3	4,0	6,2
10	Yes	11,3	4,6	19,2 A	2,6	2,0	2,9	21,6	8,7	37,1	5,0	3,9	5,4
20	Yes	11,5	4,1	18,6	2,1	1,9	2,2	58,6	21,0	95,0	10,3	9,6	11,2
50	Yes	19,1	5,5	21,2 A	2,7	2,2	2,9	74,9	21,7	83,5	10,7	8,5	11,5
100	Yes	31,8	6,8	18,2	2,4	2,2	3,2	107,3	22,9	62,2	8,4	7,4	10,7
5		8,8	5,6	19,8	3,5	2,4	3,3	14,9	9,6	34,1	5,9	4,2	5,6
10		9,9	4,6	18,2	2,5	2,0	2,7	22,7	10,6	42,6	6,0	4,8	6,2
20		11,3	4,2	19,1	2,2	1,8	2,3	59,8	22,0	101,5	11,5	9,6	12,2
50		19,0	5,3	20,1	2,4	2,1	2,6	77,0	21,2	81,1	9,5	8,4	10,4
100		30,1	6,8	19,1	2,4	2,2	3,3	110,6	25,1	71,5	8,9	8,1	12,0
Control		14,9 B	5,1	18,9	2,4 B	2,0	2,6	58,4	18,5	70,2	8,6	7,3	9,6
N foliar		16,7 A	5,5	19,7	2,8 A	2,2	3,0	55,6	16,9	62,1	8,1	6,7	9,0
p N supply		<0,01*	<0,01*	0,14	<0,01*	<0,01*	0,04*	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*
p N foliar		0,02*	0,11	0,11	0,06*	0,18	0,09*	0,49	0,21	0,15	0,44	0,27	0,53
p N supply x N spray		0,43	0,22	0,03*	0,11	0,81	0,18	0,98	0,59	0,69	0,18	0,7	0,4

N supply was evaluated by the regression (p<.1); N spray was evaluated by the LSD test (p<.1). Different letters mean rates differences.

Table S5. Roots mineral contents and uptakes (N, P, K, Ca, Mg and S) by the plants under the influence of N supplies and foliar N application.

N supply	N spray	N	P	K	Ca	Mg	S	N	P	K	Ca	Mg	S
%	**-**	**---------------------------- (roots) g kg^{-1}**					**-------------------------------**	**------------------------------- (roots) g plant^{-1}**					**----------------------------**
5	No	4,5	3,8	19,9	3,2	3,0	3,8	31,6	26,0	136,3	22,7	21,0	26,5
10	No	7,3	5,0	14,0	3,5	3,6	5,6	49,1	33,5	94,9	23,1	24,0	37,1
20	No	10,3	5,7	15,2	4,3	4,8	7,2	99,6	54,9	146,6	42,7	48,0	68,9
50	No	27,9	10,5	17,6	3,5	5,2	9,7	162,3	60,8	102,1	20,4	30,6	56,0
100	No	35,4	8,9	16,0	3,2	5,2	9,3	189,8	47,3	83,7	17,0	27,7	48,7
5	Yes	5,4	3,4	16,8	2,7	2,7	3,5	26,3	16,3	80,9	13,0	13,0	16,8
10	Yes	8,2	5,6	15,9	3,9	4,2	6,7	41,8	28,6	81,4	20,0	21,5	34,4
20	Yes	11,2	6,6	17,4	5,1	5,6	8,3	85,8	50,8	134,7	38,9	43,1	65,0
50	Yes	32,7	9,2	16,9	3,5	4,9	8,3	167,7	47,3	87,5	17,7	25,0	43,0
100	Yes	34,4	9,5	18,5	3,0	4,7	9,6	195,0	54,0	104,4	17,3	26,4	54,1
5		5,0	3,6	18,4	3,0	2,9	3,7	29,0	21,2	108,6	17,8	17,0	21,6
10		7,8	5,3	14,9	3,7	3,9	6,1	45,4	31,0	88,1	21,6	22,7	35,7
20		10,7	6,1	16,3	4,7	5,2	7,8	92,7	52,8	140,6	40,8	45,5	67,0
50		30,3	9,9	17,3	3,5	5,0	9,0	165,0	54,0	94,8	19,0	27,8	49,5
100		34,9	9,2	17,2	3,1	4,9	9,4	192,4	50,7	94,0	17,1	27,1	51,4
Control		17,1 B	6,8	16,5	3,5	4,4	7,1	106,5	44,5	112,7	25,2	30,3	47,4
N foliar		18,4 A	6,9	17,1	3,6	4,4	7,3	103,3	39,4	97,8	21,4	25,8	42,6
p N supply		<0,01*	<0,01*	0,52	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*
p N foliar		0,09*	0,87	0,66	0,53	0,89	0,77	0,63	0,05*	0,12	0,09*	0,08*	0,19
p N supply x N spray		0,21	0,30	0,59	0,15	0,1	0,44	0,85	0,13	0,17	0,66	0,92	0,54

N supply was evaluated by the regression (p<.1); N spray was evaluated by the LSD test (p<.1). Different letters mean rates differences.

EFFECTS OF FOLIAR APPLICATION OF NITROGEN (^{15}N) AND MOLYBDENUM ON NITRATE AND AMMONIUM METABOLISM IN SUGARCANE CULTIVATED UNDER NITROGEN SUPPLY LEVELS

Abstract

Foliar fertilization is an important tool for sustainable crop management. The effectiveness of foliar fertilization depends on several factors, the nutritional status of the plants being one of the principals. As sugarcane is a crop with low nitrogen use efficiency (NUE), foliar fertilization becomes an important tool to improve the NUE, in addition to nutrients directly involved in N assimilation, such as Molybdenum (Mo). An experiment in nutrient solution was conducted to assess the effectiveness of N and Mo applied through the leaves in plants growing under N and Mo deficiency. The experiment was conducted as a factorial 2 x 2 x 2, with 2 supplies of N, 0.75 mM N (low) and 3.0 mM N (adequate), with (0.75 mg N plant^{-1}) or without N foliar application, and with (4.5 mg Mo plant^{-1}) or without Mo foliar application. The effects on dry matter and mineral contents in the plant compartments, ^{15}N recovery, and the NH_4^+ and NO_3^- contents were evaluated. Plants growing under adequate N supply increased biomass production by 194% and N accumulation by 372% compared to N-deficient plants. Foliar application of N resulted in an increase of 5.1% in biomass production, but no interaction with Mo was observed. Bottom leaves of N-adequate plants showed less dry mass and lower levels of ammonium with the use of Mo, compared to the plants without Mo, indicating an acceleration in the production of new shoots. The recovery of foliar applied N was 29.5 and 36% respectively for N-deficient and adequate plants. N-deficient plants showed 68% more remobilization of foliar spray N-urea to the roots than the N-adequate plants. N-adequate plants utilize N and Mo applied more effectively, allocating these nutrients to the new shoots.

Keywords: foliar fertilization, Nutrient solution, sustainable crop management, Nitrogen deficiency, nutrient uptake, nitrogen use efficiency.

2.1 Introduction

Foliar fertilization with one or more nutrients is often used to complement soil fertilizer applications (Oosterhuis, Weir, 2009). This technique can improve nutrient use efficiency (NUE) and quickly supply urgently needed nutrients for adequate plant growth and high yield (Habib, 2012; Oosterhuis, Weir, 2009). Among the foliar nitrogen (N) fertilizers, urea is commonly used due to its high foliar absorption rate and low cost (Witte et al., 2002). Foliar N application is typically performed when the optimal period for successful soil application has passed (Gerik et al., 1998).

Plant nutritional status, crop development stage, leaf age, leaf surface characteristics, and climatic circumstances at the time of application are variables that might affect how effective foliar N fertilization will perform (Fageria et al., 2009; Fernandez, Eicherr, 2009). In plants with low leaf N concentration, foliar-applied N-urea is more readily absorbed and translocated, according to studies on hydrangea, apple, and citrus (Cheng et al., 2002; Bi and Scagel, 2008; Lea-Cox and Syvertsen, 1995). In contrast, a higher cuticular wax concentration in plants with nutritional deficiencies causes foliar N uptake to be lower (Bondada et al., 2001; Klein and Weinbaum, 1984).

Foliar fertilization is a promising way to increase NUE in sugarcane as the crop exhibited a low NUE. The crop recovery of N-fertilizer averages only 26% of the N applied, while 32% was found immobilized into soil organic matter, and the remainder being susceptible to losses such as volatilization, denitrification, or leaching (Otto et al., 2016). The effect of foliar N application on sugarcane has been investigated in the past, more specifically in 1980s with promising results (Trivelin et al., 1988). The application of foliar N spray in sugarcane has become a common practice since then, but with little scientific validation.

The nutritional status of other nutrients can influence the absorption and translocation of N foliar applied. The absorption and translocation of foliar -applied N-urea in *Camellia sinensis* L. were studied under N, potassium (K), magnesium (Mg), and sulfur (S) deficiency (Ruan and Gerendas, 2015). However, there are no studies on the effect of foliar N application in plants with micronutrient deficiencies, especially with molybdenum (Mo), which is a nutrient co-factor of the enzyme nitrate reductase, which participates in the assimilation of N in ammoniacal forms (Campbell, 1999).

A series of experiments recently conducted showed a significant response of sugarcane to the soil application of micronutrients in the planting furrow (Mellis et al., 2016). On this study, Zn and Mo presented the most significant effects, with productivity gains of 19 and 12 Mg ha^{-1}, respectively, on average of eleven experiments performed under field conditions. Based on this dada, the recently launched edition of official recommendation bulletin of São Paulo State, the main producer of sugarcane in Brazil, recommends Mo application in both sugarcane planting and ratoon crops (Cantarella, et al., 2022). However, the adoption of Mo application by growers depends on an effective way of supplying this element in fertilization programs. A foliar application of Mo may be an alternative, considering the high price of the nutrient and the low rates usually applied.

This study tested the hypothesis that the combined supply of N and Mo through foliar application increases NUE compared to the isolated application of N. The objective was to evaluate the effect of N and Mo foliar applied on nutrition, N metabolism, ^{15}N recovery, and sugarcane biomass production under controlled conditions.

2.2 Material and Methods

2.2.1 Site and material characterization

The experiment was carried out in a growth chamber at the Department of Soil Science at ESALQ/USP. The growth chamber was maintained at constant temperature of 32 $\pm$ 2 °C during daylight hours and 18 $\pm$ 2 °C at nighttime throughout the duration of the experiment, which had a 13-hour photoperiod. Throughout cultivation, the relative humidity in the growth chamber was kept at 80%.

Sugarcane seedlings were obtained from two-centimeter-long stalks pieces (containing one bud per stalk piece) of a nine months old plant. The RB966928 variety was chosen because its representative sugarcane cultivation area in Central South Brazil. The stalk pieces with one bud were then treated with fungicide and put in plastic trays with washed sand as the substrate (Figure 2, supplementary A). The seedlings were irrigated daily with deionized water and, twenty-one days after planting, thirty seedlings of uniform size were chosen to be transplanted into the nutrient solution pots.

The pots and lids were sprayed with silver spray to keep the nutritional solution's temperature from rising, and Styrofoam plates were set on the bench to soak up any additional heat (Figure 2, supplementary B). For cultivation, Hoagland and Arnon's solution (1950) was used, where 7.5 mM NH_4NO_3 (100%), 1 mM KH_2PO_4, 2 mM $MgSO_4$, 5 mM $CaCl_2$, 5 mM KCl, 3.5 mM K_2SO_4 were supplied and the micronutrients: H_3BO_3 (0.5 µM), $CuSO_4.5H_2O$ (0.02 µM), Fe-EDTA (5 µM), manganese $MnSO_4.H_2O$ (0,5 µM) and zinc $ZnSO_4$ (0.05 µM). Mo was omitted from the nutrient solution.

To allow the seedlings to adjust to their new surroundings, the nutrient-first solution was created with only 50% of the intended ionic strength. The nutrient solution was made with 100% of ionic strength at the first solution change, which occurred seven days following installation. Five changes were made to the answer over the course of the test, one every week. Every day, 0.5 M HCl or 0.5 M NaOH was used to bring the pH of the solution down to 5.8.

2.2.2 Experimental design and foliar application

The experiment was designed using randomized blocks in a triple factorial scheme (2x2x2), as follows: 2 supplies of N (0.75 and 3.0 mM), 2 foliar N application (control, without application, and with the supply of 0.75 g plant^{-1} N), and 2 levels of Mo foliar application (control and 4.5 mg plant^{-1} Mo) (Figure 1). The N source used for foliar spray was urea labeled ^{15}N (excess of 2.07% atoms of ^{15}N) and the Mo source was ammonium molybdate.

	T1	T2	T3	T4	T5	T6	T7	T8
Factor 1 (N supply)	Low	Low	Low	Low	Adequate	Adequate	Adequate	Adequate
Factor 2 (^{15}N foliar)	Without	Without	With	With	Without	Without	With	With
Factor 3 (Mo foliar)	Without	With	Without	With	Without	With	Without	With

Figure 1. Details of treatments and experimental scheme.

The plants growth under 0.75 mM of N supply were referred to as N-deficient and the plants grown under 3.0 mM of N supply was referred to as N-adequate (based on Chapter 2). The foliar application was performed using 1.5 ml of a solution with 0.1% tween 20, and 5% of N-urea, totalizing 75 mg plant^{-1}. Labeled urea (2.07% atom ^{15}N) was diluted into deionized water prior to application. The three youngest leaves of the sugarcane plantlets were exposed to the N solution on both their abaxial and adaxial surfaces, using a brush, registering the exactly amount of solution applied in each plant by means of weighing in a scale (0.001 kg error) the brush before and after the application. For identification purposes, a string was used to indicate the leaves that received foliar N spray.

2.2.3 Measurements

The plants were cultivated for 30 days prior to the foliar treatment. After foliar N and Mo application, plants were grown for more seven days before measurements. At harvesting time, the biomass of stems, roots, treated and untreated leaves were quantified.

In order to remove urea adhered to leaf surface, the leaves were washed in the following sequence: distilled water with 1 ml l^{-1} of neutral detergent, solution with 0.1 M of HCl and distilled water. Samples from all plant compartments were dried in a forced air circulation laboratory oven at 65 ◦C during 72h, and ground in a Wiley-type mill. Care was taken to clean the mill between samples in order to avoid cross contamination with ^{15}N. Samples from all plant compartments were split into three samples: one for quantification of mineral nutrients contents; other for quantification of nitrate and ammonium contents, and the remaining sample for analysis of total N and ^{15}N abundancy.

The mineral contents (N, P, K, Ca, Mg, S, Fe, Zn, Mn, Cu, and B) were determined according to the methodology described by Malavolta, Vitti and Oliveira (1997). Molybdenum content was determined using the same procedure adopted by Mellis et al. (2016).

Leaf ammonium and nitrate contents in plant tissues were determined using the methodology described by Tedesco, Volkweiss and Bohnen (1985). Inorganic N was extracted through a 1M KCl solution, in which 1 g of dry plant tissue was mixed with 15 mL of extracting solution (ratio plant: solution 1:15 - m/v). After shaking the samples with KCl and filtering the suspension, ammonium was quantified by distillation with MgO, whereas nitrate was quantified after the addition of Devarda's alloy in the same extract used for ammonium quantification. Both inorganic N fractions were quantified in the extract by titration with 0.0025 M H_2SO_4 solution.

The total N content and the $^{15}N/^{14}N$ isotopic ratio (atom % ^{15}N) were determined in a Hydra 20-20 SerCon Co., UK mass spectrometer, coupled to an ANCA-SGL N automatic analyzer (BARRIE; PROSSER, 1996). Using the atoms % ^{15}N in the sample and the amount of N in each compartment of the sugarcane (g plant), it was possible to calculate the amount of N derived from fertilizer (NDFF) and ^{15}N recovery (R)using the following equations.

$$NDFF\ (\%) = \left[\frac{a-b}{c-b}\right] x\ 100$$

a is the abundance of ^{15}N % atoms in the sample, *b* is the natural abundance of ^{15}N % atoms (0.366%), *c* is the abundance of ^{15}N % atoms in the fertilizer (2.07 % atom ^{15}N).

$$R(\%) = \left[\frac{\frac{NDFF\ (\%)}{100}\ x\ NA(mg\ plant)}{N\ rate\ (mg\ N\ plant)}\right] x\ 100$$

NDFF is the amount of N derived from fertilizer (%), *NA* is the amount of N accumulated in each plant compartment (mg plant), *N rate* is the amount of foliar applied N (75 mg $plant^{-1}$)

2.2.4 Statistical analysis

The Shapiro-Wilk and Levene tests (P>0.1) were used to assess the assumptions of normality and variance homogeneity. When Grubbs' test detected outliers, they were removed. The F-test was used to submit data to ANOVA. When the F-test was significant (P< 0.05), the LSD test (P<0.1) was performed to compare interactions since it is a pairwise comparison test and the unfolding of interactions has only one degree of freedom.

2.3 Results

2.3.1 Dry mass and nutrients uptake

All plants compartments grown with adequate N produced a higher amount of dry mass compared to plants grown with low N. Plants supplied with adequate N had a dry mass of 29.3 g plant^{-1}, while low N had a total mass of 15.1 g plant^{-1}, that is, plants supplied with adequate N presented 94% more biomass production compared to plants supplied with low N (Figure 2).

N-adequate plants without foliar Mo produced 26% more dry mass of old leaves than treated plants, and this effect was not detected in N-deficient plants. Plants grown in N-deficient nutritive solution treated with N and Mo through the leaves had a lower dry mass of roots compared to other plants in the same N supply. N foliar application resulted in a significant increase of 5.1% in dry biomass, and no interaction with Mo was observed (Figure 2).

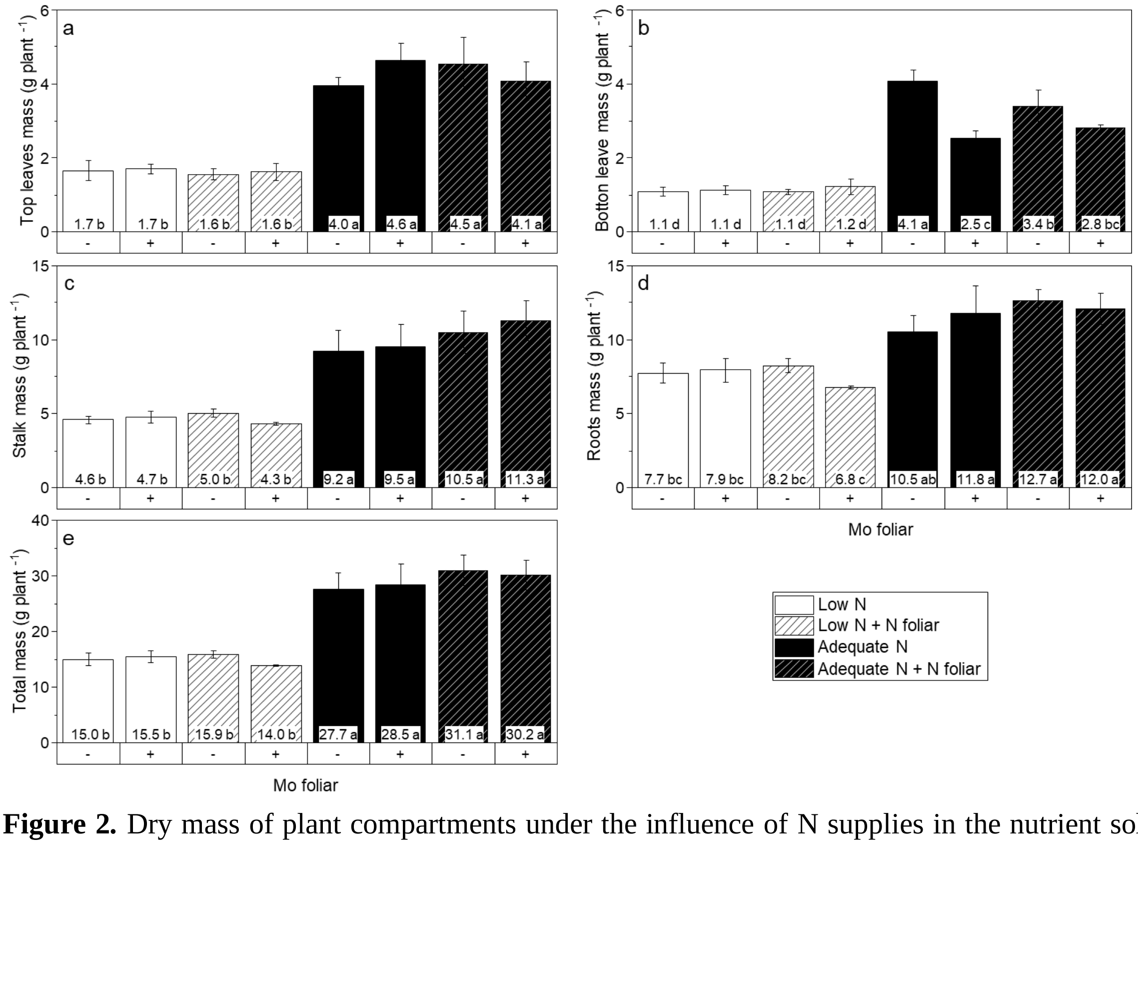


Figure 2. Dry mass of plant compartments under the influence of N supplies in the nutrient solution,

N and Mo foliar treatments. **a**, top leaves dry mass; **b**, botton leaves dry mass; **c**, stalks dry mass **d**, roots dry mass; **e,** total dry mass. Means were compared by LSD test using $p < 0.05$.

The was a significant effect of N supply in the nutrient solution in N accumulation in plant compartments, whereas N and Mo applied through the leaves showed no consistent effects. N-adequate plants presented an N accumulation of 408 mg plant^{-1}, while N-deficient plants accumulated 109 mg plant^{-1}. On average, plants treated with foliar N spray presented N accumulation 31 mg plant^{-1} N higher compared to plants not receiving foliar N (TABLE S2). In plants cultivated with low N content, there was no effect of foliar treatments on N accumulation in any compartment.

Plants grown with adequate N and treated with foliar N, showed no significant effect on the uptake of N in plant compartments. Plants grown with N-adequate and with Mo foliar application generated less N accumulation on the bottom leaves than the non-treated plants (Figure 3).

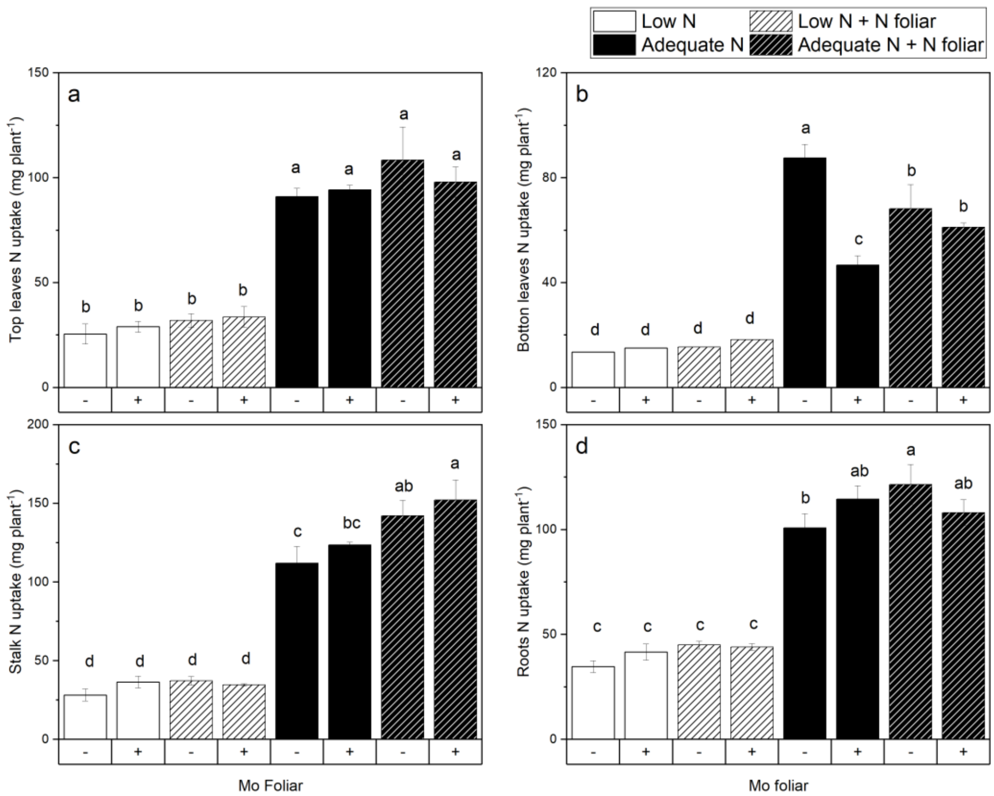


Figura 3. N absorption in plant compartments. **a**, N uptake on top leaves; **b**, N uptake in botton leaves; **c**, N uptake in stalk, **d**, N uptake in roots. Means were compared by LSD test using $p < 0.05$.

Foliar spray of Mo has a pronounced effect on the accumulation of Mo in plant tissues. Plants treated with foliar Mo had an total Mo uptake of 1,230 µg plant^{-1}, while untreated plants had 39 µg plant^{-1} Mo (Figure 4). The top leaves, i.e., leaves that received the application, of N-adequate plants, showed higher Mo uptake compared to N-deficient plants (Figure 4).

Mo uptake in bottom leaves only occurs for plants that received foliar N treatment together, i.e., there was Mo remobilization only when it was applied together with N-urea. The highest N uptake in stalks was observed in the plant treated with foliar N and Mo in adequate N supply. The roots of N-deficient plants treated with Mo showed higher levels of Mo accumulation compared to plants grown in N-adequate (Figure 4).

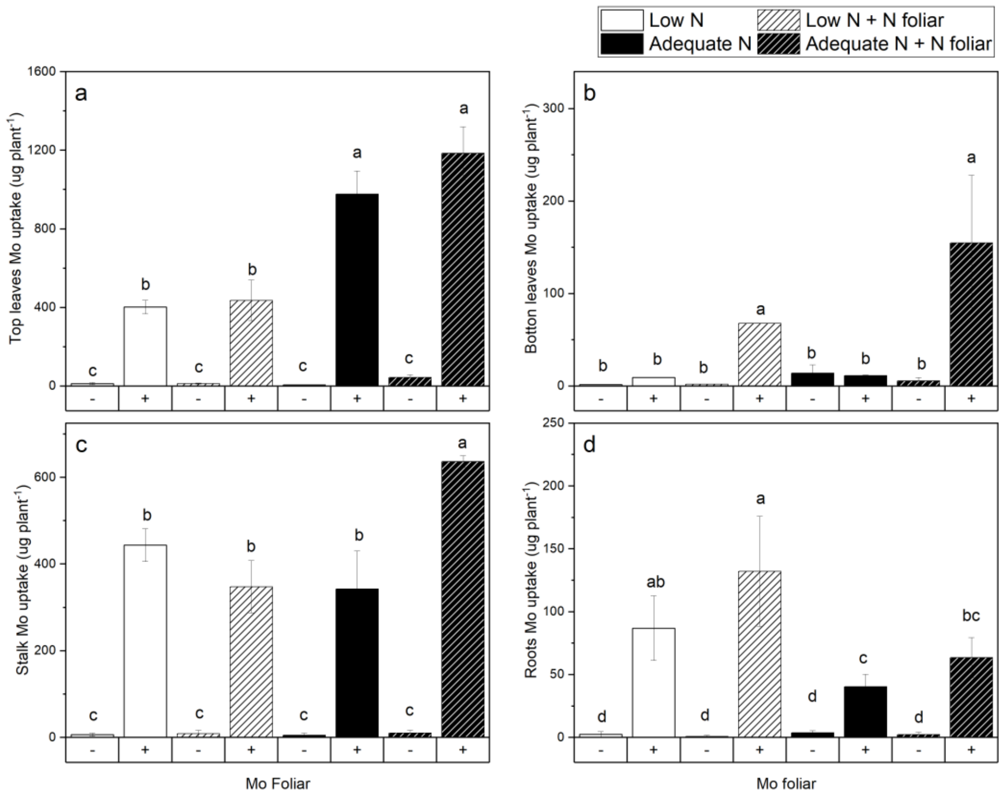


Figura 4. Mo absorption in plant compartments. **a**, Mo uptake on top leaves; **b**, Mo uptake in botton leaves; **c**, Mo uptake in stalk, **d**, Mo uptake in roots. Means were compared by LSD test using $p < 0.05$.

2.3.1 Nitrate and ammonium contents

In the top leaves of N-deficient plants treated with foliar N, N-NO_3 levels were higher compared to plants not treated with foliar N. The Mo application reduced the N-NO_3 in the top leaves of N-adequate plants. The N-NH_4^+ content in the top leaves was higher in plants treated with N and Mo foliar (Figure 5).

In the bottom leaves, for N-deficient plants, the N-NO_3 content was higher in the treatment with foliar N and Mo, whereas for the N-adequate plants, the N-NO_3 content was higher in the plants without foliar treatment. The N-NH_4^+ bottom leaves content was higher in N-deficient plants treated with Mo, the opposite was observed in plants grown in adequate N, where the N-NH_3 content was lower with Mo application (Figure 5).

The stalks of N-adequate plants showed significant increments of 66 and 82% of average levels of N-NO_3 and N-NH_4^+, respectively, compared to N-deficient plants (Table S6). The N-adequate plants without any foliar treatment showed the highest N-NO_3 contents in all compartments compared to the other treatments. The Mo foliar application reduced the N-NH_4^+ contents in the stalks of N-adequate plants. There was no N-NO_3 and N-NH_4^+influence of foliar treatments on stems of N-deficient plants.

The roots of N-deficient plants showed, on average, lower N-NO_3 contents and higher N-NH_4^+contents, compared to plants cultivated with adequate N. In N-deficient plants treated with foliar N, N-NH_4^+contents were 131% higher compared to the average of plants without foliar treatment (Table S5).

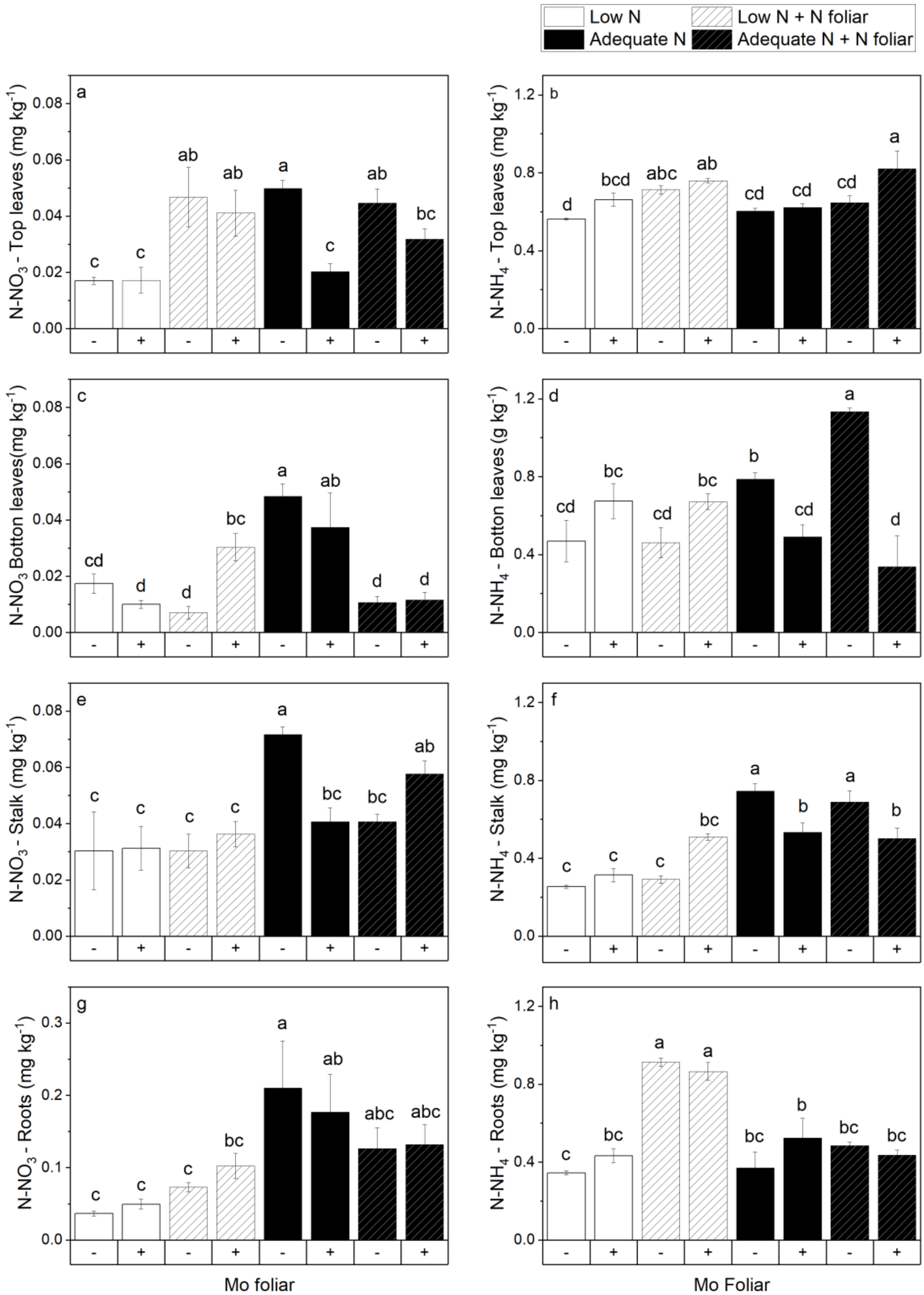


Figure 5. Nitrate and ammonium concentration in plant compartments. **a**, N-NO_3 content in top leaves; **b**, N-NH_4^+content in top leaves; **c**, N-NO_3 content in bottom leaves; **d**, N-NH_4^+content in bottom leaves; **e**, N-NO_3 content in the stem; **f**, N-NH_4^+content in the stem; **g**, N-NO_3 content in the roots; **h**, N-NH_4^+content in the roots. Means were compared by LSD test using $p < 0.05$.

2.3.2 Nitrogen derived from fertilizer and ^{15}N recovery

The percentage of N derived from the foliar fertilizer (NDFF) in the tissues of the N-deficient plants was higher in all plant compartments compared to the N-adequate plants. The highest NDFF was found in the top leaves, this compartment received the foliar treatment directly. For N-deficient and N-adequate, the top leaves recovered 11 and 19% of the N applied on the leaves, respectively. Mo treatment aided foliar N recovery in plants cultivated in adequate N (Figure 6).

Recovery of N-fertilizer by plant compartments followed the order Top Leaves > Stalks > Roots > Old leaves for all treatments. Old leaves recovered only 2.8% of applied N on average, and there was no difference between the N level in the crop or the Mo treatment. There was also no effect of foliar N and Mo on the N fertilizer recovery by top leaves, and the average recovery in this compartment was 10.1%. The roots of N-deficient plants recovered, on average, 5.9% of N applied to the leaf, 68% more compared to plants grown in adequate N (Figure 6).

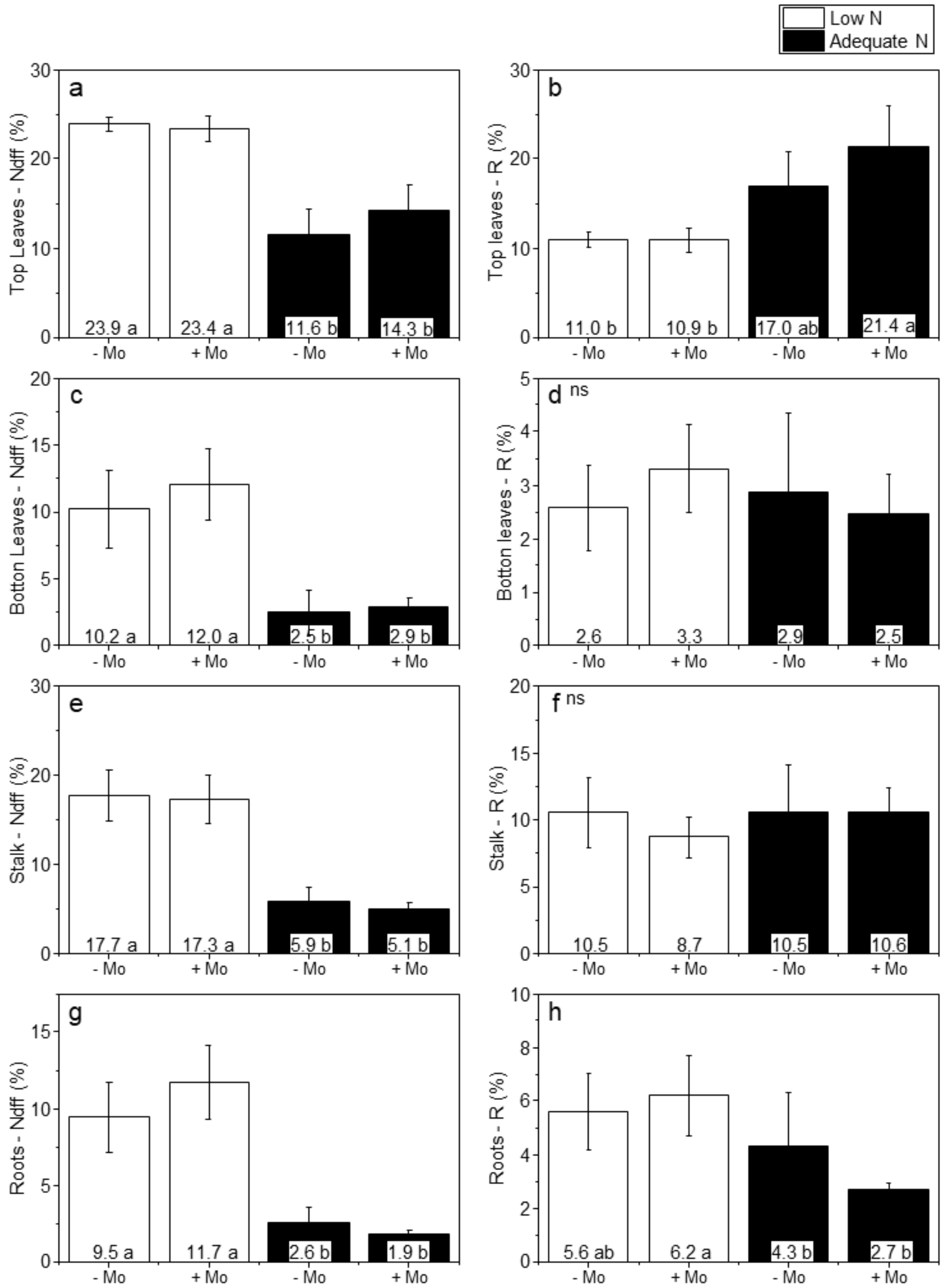


Figure 6. Nitrogen in the plant derived from fertilizer (NDFF) and total ^{15}N recovery (R) in plant compartments. **a**, NDFF in top leaves; **b**, N Recovery in top leaves; **c**, NDFF in bottom leaves; **d**, N Recovery in bottom leaves; **e**, N in the stalk; **f**, N Recovery in the stalk; **g**, NDFF in the stalk; **h**, N Recovery in the roots. Means were compared by LSD test using $p < 0.05$.

The total N recovery of foliar applied fertilizer was obtained in N-adequate plants, reaching up to 37.2% of total recovery. The N-deficient plants presented lower recovery of the applied N, on average 29.4 in the applied N (Figure 7).

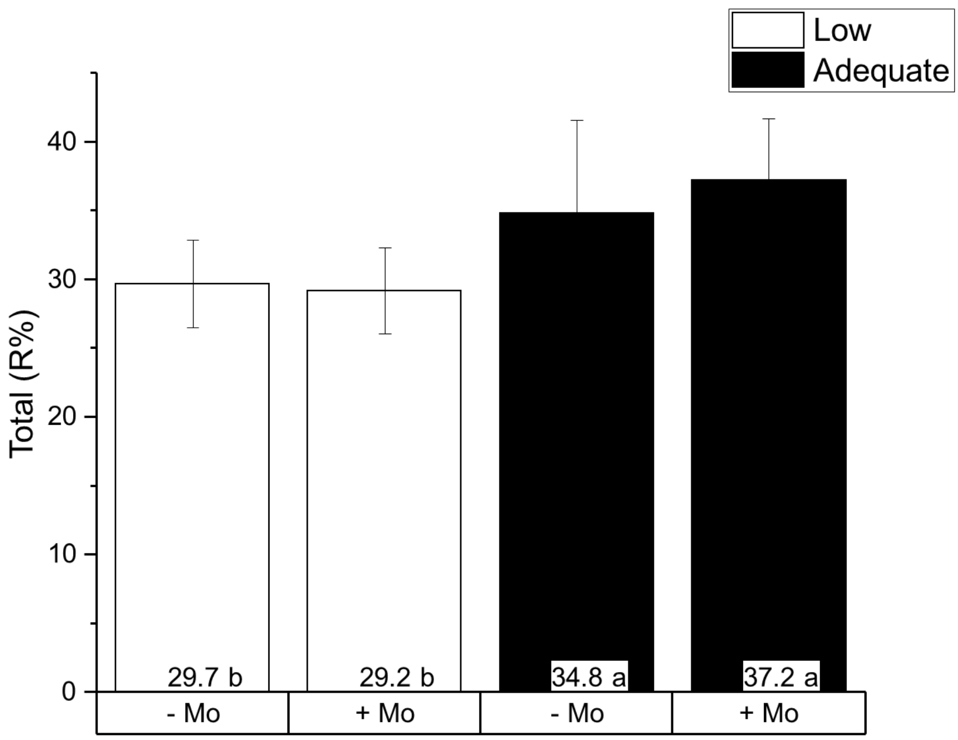


Figure 7. Total nitrogen foliar applied recovery (%). Means were compared by LSD test using $p < 0.05$.

2.4 Discussion

As expected, adequate N supply in the solution promoted more robust growth and higher biomass production in all compartments (Figure 2). Foliar N application resulted in a significant increase of 5.1% in dry biomass (Table S1) and Mo showed no effect on biomass production. Considering the evaluation occurred 7 days after Mo treatment, the biomass accumulation response may have been limited. Plants that just received Mo foliar treatment generated more biomass than control plants, but when the application was combined with N, the result was the opposite. Although there was no statistical difference, it is possible that the N metabolism was overloaded when the application was combined.

N-adequate plants with foliar Mo had 26% dry mass of old leaves compared to plants treated with foliar Mo. This suggests that the application of foliar Mo may have negatively influenced dry mass accumulation in old leaves of plants grown in adequate N. It is possible that the Mo accelerated the assimilation of N in the treated leaves and then the old leaves could have become a sink for photo assimilates.

Plants cultivated in adequate N solution accumulated 374% more N and 194% more dry mass compared to plants grown in N-deficient nutritive solution. Thus, plants cultivated in low N had an average concentration of 7.2 mg N g^{-1} plant, while plants grown in adequate N had 13.9 mg N g^{-1} plant. That is, plants with a high N supply produced almost twice as much dry mass and four times as much N uptake, due to the abundance of N in the nutrient solution. Plants that received foliar N application, with 75 mg $plant^{-1}$ N, on average had 31 mg of N more than untreated plants, most of this N coming from the foliar application (Table S2).

The total amount of Mo applied was 4,500 µg $plant^{-1}$ Mo, equivalent to 300 g ha^{-1} Mo, the optimal rate found in the work by Mellis, et al. (2016). Considering that the average of the plants that received the foliar application contained 1,320 µg $plant^{-1}$ Mo and the untreated plants 39 µg $plant^{-1}$ Mo, the average absorption of the treated plants was 28% of the Mo applied rate (Table S2). Since the experiment was carried out in the absence of Mo in the nutrient solution, it is feasible to conclude that in the Mo deficient treatments, the plant can absorb and transport the Mo leaf applied rapidly. Recent advances are elucidating how Mo is transported in plants, and affinity and non-specific transporters have already been found. As the molybdate shares similar biochemical properties with sulfate, allowing it to be taken up by the sulfate transport system in a non-specific manner (Huang and Zhao, 2021).

The N-deficient plants had 934 ug of Mo on average, while the N-adequate plants contained 1,705 ug of Mo. Plants grown in enough N may have higher plant absorption as well as higher leaf area and cell membrane integrity. The nutritional plant status influenced Mo translocation. N-deficient plants translocated more Mo to roots compared to plants grown in adequate N supply. On average, plants with low N supply accumulated 109.5 mg $plant^{-1}$ Mo in the roots while N-adequate plants 51.4 mg $plant^{-1}$ Mo. Therefore, there was a greater ability to transport Mo in N-deficient plants. Possibly the N-deficient plant activated mechanisms to produce Mo transporters due to the lack of N and low assimilation of nitrate from the roots.

Some authors described the transcription of CrMOT2, which is a molybdate transporter, is induced by molybdate deficiency and connected to nitrate reductase activities, this may explain why N-deficient transported more Mo to roots than the N-adequate plants (Tejada-Jimenez et al., 2007; Tomatsu et al., 2007).

Mo translocation to bottom leaves was increased when done in conjunction with N-urea application. Plants treated with only Mo did not translocate Mo to old leaves, which may indicate a synergism in the foliar application of N and Mo. The facilitation of Mo translocation may be associated with support in the velocity of N absorption provided by the effect of breaking the surface tension caused by urea. The extent and rate of Mo translocation may vary depending on the plant species, environmental conditions, and the way in which the micronutrient is applied.

In N-deficient plants, a significant increase in nitrate and ammonium contents was observed in young leaves when submitted to N foliar treatment. Application of urea, i.e., a source of ammoniacal N, was expected to result in an increase in ammonium levels in leaves. However, the increase in nitrate levels may be related to the translocation of ammonium to the roots and, consequently, to an increase in the influx of nitrate in these leaves. This condition suggests the existence of interactions and transport processes between ammonium and nitrate, where the presence of ammonium in the roots can stimulate the influx of nitrate in the leaves, even in N deficiency condition.

In the top leaves of N-adequate plants, the Mo application resulted in a reduction in nitrate concentration. This reduction suggests an increase in the activity of the enzyme nitrate reductase, responsible for converting nitrate into nitrite and, subsequently, into ammonium. Therefore, the presence of Mo could stimulate nitrate reductase activity, leading to a decrease in nitrate levels in leaves. Regarding the ammonium content, an increase was observed only in the new leaves of the plant treated with leaf N and Mo. This indicates that the combined application of N and Mo foliar applied results in a specific increase in the ammonium content in these leaves.

In the bottom leaves of N-deficiency plants, an increase in ammonium levels was observed with the application of Mo (Figure 5). This indicates that the presence of Mo affected N metabolism in these leaves, resulting in increased ammonium accumulation. Furthermore, in the old leaves of N-deficient plants, there was an increase in nitrate levels when submitted to the combined treatment of N and Mo. This indicates that the combined application of N and Mo results in a specific increase in nitrate levels in these leaves.

In the bottom leaves of plants cultivated with adequate levels of N, it was observed that N foliar treatment resulted in a decrease in the amount of nitrate. This reduction may be related to the stimulus to produce new leaves since nitrate is mobilized to growing tissues. Furthermore, in the stalks of plants grown in adequate N, treatment with Mo resulted in a decrease in ammonium concentration. In the stalks of N-deficiency plants, no significant effect was observed on nitrate levels with foliar treatments. As for the plants grown in adequate N, the treatment with Mo helped in the ammonium reduction.

According to the results, the roots of the N-deficient plants that received the foliar N treatment showed a concentration of ammonium twice as high compared to the untreated plants. This indicates that there was a significant translocation of ammoniacal N, from the application of urea on the leaves to the roots.

The amount of N derived from fertilizer (NDFF) was higher in all compartments of the N-deficient plants compared to the plants supplied with adequate levels of N. This occurred because the N-deficient plants had a lower amount of dry mass and less accumulation of N in tissues compared to plants supplied with sufficient N. Young leaves were the compartments that showed the highest NDFF values since they received the direct application of foliar fertilizer.

Trivelin et al. (1988), using ^{15}N-urea, found that more than 50% of the foliar-applied urea (82 mg plant^{-1} N) was rapidly absorbed by the sugarcane within 6 hours. However, no substantial increase in foliar absorption was detected in the following days. Washing the plant shoots after the harvest had a substantial influence on fertilizer recovery, with around 5% of the absorbed N translocated and recovered in the sugarcane root system after 96 hours. In our study, using 75 mg plant^{-1} N, we found that the average absorption of 33% of the N foliar-applied. Since the growing chamber was kept humid with nebulizers, the leaves were rehydrated to simulate dew, i.e., unabsorbed N can be lost by volatilization due to urease activity on leaves. This fact is supported by evidence from the work of Trivelin et al. (1988), who observed there is no absorption after 6 hours of application, even after rehydrating the leaves.

The N-deficient plants presented a recovery rate of 29.5% of the applied N, regardless of Mo application. It was observed that top leaves from N-deficient plants had a lower recovery of applied N, which can be attributed to their smaller leaf area compared to N-adequate plants. N-deficient plants recovered significantly more fertilizer N in the roots compared to N-adequate plants. This suggests that under conditions of N deficiency, the roots can act as an important sink of N and can be an adaptive response of the roots as a strategy to compensate the nutritional deficiency.

Plants cultivated at adequate N, showed an average rate of recovery of 36% of the applied N, with approximately 30% of this N being retained in the leaves that directly received the application and in the stalks. The stalks showed an average N recovery of 11%, which would probably be directed towards the development of new leaves.

Ruan and Gerandás (2015), studying the foliar applied urea-^{15}N in *Camellia sinensis* L., found that the efficiency of foliar N was reduced under depleted N, K, and S nutritional status, which weakened the N sink strength due to impaired young shoot growth instead of old shoot and roots. The authors found that the variation in total ^{15}N absorption was mostly due to the component in immature shoots, with no significant differences in ^{15}N levels in mature leaves and stems.

According to Bondada et al. (2001), N-deficient citrus leaves exhibited lower foliar N absorption than N-sufficient leaves. This difference in N absorption was attributed to an increase in epicuticular wax concentration in the N-deficient leaves. The wax characteristics of the leaves were not investigated in this study, but another parameter that can be considered is more leaf area in the N-adequate plants.

These results indicate that plants with adequate N nutrition have a greater capacity to utilize and efficiently allocate absorbed N. The greater retention of N in the leaves that received the direct application and, in the stalks, suggests that these parts of the plant are important reservoirs of N for subsequent growth and development.

2.5 Conclusion

Plants growing under adequate N supply increased biomass production by 194% and N accumulation by 372% compared to N-deficient plants. Foliar application of N resulted in an increase of 5.1% in biomass production, but no interaction with Mo was observed. Recovery of Mo applied averaged 28% of the applied rate, and the contents of Mo in plant compartments increased sharply after a seven days application period.

The recovery of foliar applied N was 29.5 and 36% respectively for N-deficient and N-adequate plants, demonstrating that well-nourished plants are more able to better use foliar N spray. N-deficient plants showed 68% more remobilization of foliar spray N-urea to the roots than the N-adequate plants. N-adequate plants utilize N and Mo foliar applied more effectively allocating the N and Mo to the new shoots.

These findings have significant implications for optimizing foliar fertilization in nutritional management, where we have strong evidence that the highest returns with N and Mo foliar fertilization occurred in well-nourished plants.

References

BARRIE, A.; PROSSER, S. J. Automated analysis of light-element stable isotopes by isotope ratio mass spectrometry. p. 1-46. In: Boutton T.W.; Yamasaki S.I., eds. Mass spectrometry of soil. Marcel Dekker, New York, NY, USA, 1996

BI, G.; SCAGEL, C. F. Nitrogen uptake and mobilization by Hydrangea leaves from foliar-sprayed urea in fall depend on plant nitrogen status. **Horticultural Science**, v. 43, p. 2151–2154, 2008.

BONDADA, B. R.; SYVERTSEN J. P.; ALBRIGO, L. G. Urea nitrogen uptake by citrus leaves. **Horticultural Science**, v. 36, p. 1061–1065, 2001.

CAMPBELL, M. K.; FARRELL, S. O. **Bioquímica**. São Paulo: Cengage Learning, 2016.

CANTARELLA, H.; QUAGGIO, J. A.; MATTOS JR, D.; BOARETTO, R. M. RAIJ, B. V. Boletim 100: **Recomendações de adubação e calagem para o estado de São Paulo**. Instituto Agronômico de Campinas, 2022, 581p.

FAGERIA, N. K.; BARBOSA FILHO, M. P.; MOREIRA, A.; GUIMARÂES, C. M. Foliar fertilization of crop plants. **Journal of Plant Nutrition**, v. 23, p. 1044-1064, 2009.

FERNÁNDEZ, V.; EICHERT, T. Uptake of hydrophilic solutes through plant leaves: current state of knowledge and perspectives of foliar fertilization. **Critical Reviews in Plant Science**, v. 28, p. 36–68, 2009.

HABIB M. Effect of supplementary nutrition with Fe, Zn chelates and urea on wheat quality and quantity. African Journal of Biotechnology, v. 11, p. 2661-2665, 2012.

HOAGLAND, D.; ARNON, D. I. **The water culture method for growing plants without soil.** California Agriculture Experimental Station, 1950, v. 347, 33 p.

HUANG, X. Y., ZHAO, F. J. Molybdenum: More than an essential element. Journal of Experimental Botany, Vol. 73, No. 6 pp. 1766–1774, 2022

KLEIN, I.; WEINBAUM, S. A. Foliar application of urea to olive, translocation of urea nitrogen as influenced by sink demand and nitrogen deficiency **Journal of American Society Horticultural Science**, v. 109, p. 356–360, 1984.

LEA-COX, J. D.; SYVERTSEN, J. P. Nitrogen uptake by Citrus leaves. **Journal of American Society Horticultural Science**, v. 120, p. 505–509, 1995.

MALAVOLTA, E.; VITTI, G. C.; OLIVEIRA, S. A. **Avaliação do estado nutricional das plantas**: princípios e aplicações. 2.ed. Piracicaba: Associação Brasileira para Pesquisa da Potassa e do Fosfato, 1997. 319 p.

MANUEL TEJADA-JIMÉNEZ, CHAMIZO-AMPUDIA, A.; GALVÁN, A.; FERNANDEZ, E.; LLAMAS, A. Molybdenum metabolism in plants, **Metallomics,** v. 5, e. 9, p. 1191–1203, 2013.

MELLIS, E. V.; QUAGGIO, J. A.; BECARI, G. R. G.; TEIXEIRA, L. A. J.; CANTARELLA, H.; DIAS, F. L. F. Effect of micronutrients soil supplementation on sugarcane in different production environments: Cane plant cycle. **Agronomy Journal**, v. 108, p. 2060–2070, 2016.

MELLIS, E. V.; KÖLLN, O. T.; MOREIRA, L. A.; OTTO, R.; FERRAZ-ALMEIDA, R.; RAMOS, L. F.; ANDRADE, R. P.; FRANCO, H. C. J. Molybdenum increases nitrogen use efficiency of sugarcane under limited N supply, **Journal of Plant Nutrition**, v. 45:9, p.1360-1369, 2022.

OOSTERHUIS, D. M.; WEIR, B. L. Foliar fertilization of cotton. In: J. M. D. Stewart, D. M. Oosterhuis, J. J. Heitholt, and J. R. Mauney, eds. Physiology of Cotton. Springer, New York, NY, USA, pp. 272– 288, 2010.

OTTO, R.; CASTRO, S. A. Q.; MARIANO, E; CASTRO, S G. Q.; FRANCO, H. C. J.; TRIVELIN, P. C. O. "Nitrogen use efficiency for sugarcane-biofuel production: what is next? **BioEnergy Research,** v. 9, p. 1272-1289, 2016.

RUAN, J; GERENDÁS, J. Absorption of foliar-applied urea-15N and the impact of low nitrogen, potassium, magnesium and sulfur nutritional status in tea (*Camellia sinensis* L.) plants. **Soil Science and Plant Nutrition**, v. 61, p. 653–663, 2015.

TEDESCO, M.J.; VOLKWEISS, S.J.; BOHNEN, H. **Análise de solo, plantas e outros materiais**. Porto Alegre: Universidade Federal do Rio Grande do Sul, 1985. 95 p. (Boletim Técnico, 5).

TOMATSU H, TAKANO J, TAKAHASHI H, WATANABE-TAKAHASHI A, SHIBAGAKI N, FUJIWARA T. 2007. An Arabidopsis thaliana high-affinity molybdate transporter required for efficient uptake of molybdate from soil. Proceedings of the National Academy of Sciences, USA 104, 18807–18812

TRIVELIN, P. C. O.; CARVALHO, J. G.; SILVA A. Q.; PRIMAVESI, A. C. P. A.; CAMACHO, E.; EIMORE, I. E.; GUILHERME, M. R. **Adubação foliar de cana-de-açúcar (*Saccharum* spp) absorção e translocação de uréia-N.** Energia Nuclear na Agricultura. Piracicaba, v. 9, p. 52-65, 1988.

WITTE, C.; TILLER, S. A.; TAYLOR, M. A.; DAVIES, H. Leaf urea metabolism in potato. Urease activity profiles and patterns of recovery and distribution of 15N after foliar urea application in wild-type and urease-antisense transgenics. **Plant Physiology** 129: 1129–1136, 2002.

Supplementary tables

Table S1. Dry mass of the plants compartments submitted to the N supply leaves and N and Mo foliar treatments

N supply	Foliar treatment		Top leaves	Botton Leaves	Stalks	Roots	Total
	N	Mo	----------	----------	g $plant^{-1}$	----------	----------
Low	-	-	1.7 b	1.1 d	4.6 b	7.7 bc	15.0 b
Low	-	+	1.7 b	1.1 d	4.7 b	7.9 bc	15.5 b
Low	+	-	1.6 b	1.1 d	5.0 b	8.2 bc	15.9 b
Low	+	+	1.6 b	1.2 d	4.3 b	6.8 c	14.0 b
Adequate	-	-	3.9 a	4.1 a	9.2 a	10.5 ab	27.7 a
Adequate	-	+	4.6 a	2.5 c	9.5 a	11.7 a	28.5 a
Adequate	+	-	4.6 a	3.4 b	10.5 a	12.6 a	31.1 a
Adequate	+	+	4.1 a	2.8 bc	11.3 a	12.0 a	30.2 a
Low			1.6 B	1.1 B	4.7 B	7.7 B	15.1 B
Adequate			4.3 A	3.2 A	10.1 A	11.7 A	29.3 A
	-		3,0	2,2	7,0	9,5	21.7 B
	Nfol		3,0	2,1	7,8	9,9	22.8 A
		-	2,9	2.4 A	7,3	9,8	22,4
		Mo	3,0	1.9 B	7,5	9,6	22,0
$p_{Nsupply}$			<0.01	<0.01	<0.01	<0.01	<0.01
$p_{Nfoliar}$			0,88	0,66	0,31	0,51	<0.01
p_{mol}			0,75	0,02	0,83	0,82	0,63
$p_{treatment}$			<0.01	<0.01	<0.01	<0.01	<0.01

Table S2. Mineral nutrient (N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, B and Mo) uptake in top leaves under the influence of low and adequate N supply, and foliar application of N and Mo. Means were compared by LSD test using $p < 0.05$.

N supply	**Foliar treatment**		**N**	**P**	**K**	**Ca**	**Mg**	**S**	**Fe**	**Mn**	**Cu**	**Zn**	**B**	**Mo**
	N	**Mo**	**------------------ Top leaves (g plant^{-1}) --------------------**						**--------------------- Top leaves (µg plant^{-1}) ----------------**					
Low	**-**	**-**	26 b	10 d	23 c	10 b	4 c	2 c	65 b	392 b	7 b	18 b	25 b	13 c
Low	**-**	+	29 b	12 d	27 c	13 b	5 c	3 c	94 b	505 b	8 b	22 b	36 b	402 b
Low	+	**-**	32 b	10 d	23 c	11 b	4 c	3 c	82 b	485 b	7 b	25 b	42 b	13 c
Low	+	+	34 b	10 d	25 c	9 b	3 c	2 c	828 a	576 b	8 b	20 b	93 ab	436 b
Adequate	**-**	**-**	91 a	29 c	50 b	17 b	9 b	8 b	218 b	448 b	23 a	68 a	57 bc	7 c
Adequate	**-**	+	94 a	42 a	81 a	40 a	13 a	10 a	297 b	869 a	27 a	65 a	118 a	997 a
Adequate	+	**-**	108 a	37 ab	87 a	39 a	12 a	11 a	287 b	902 a	28 a	69 a	130 a	44 c
Adequate	+	+	98 a	34 bc	10 ab	34 a	11 ab	9 ab	240 b	884 a	23 a	56 a	105 a	1185 a
Low			30 B	11 B	24 B	11 B	4 B	3 B	267	490 B	8 B	21 B	49 B	216 B
Adequate			98 A	35 A	72 A	33 A	11 A	9 A	260	776 A	25 A	64 A	102 A	553 A
	-		60	23	45	20	7	6	169 B	553 B	16	43	59 B	350
	Nfol		68	23	51	23	8	6	359 A	712 A	17	43	92 A	420
		-	64	21	46	19	7	6	163 B	557 B	16	45	64 B	19 B
		Mo	64	24	51	24	8	6	365 A	708 A	16	41	88 A	750 A
$p_{Nsupply}$			<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	0.93	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*
$p_{Nfoliar}$			0.10	0.77	0.34	0.28	0.70	0.37	0.02*	0.01*	0.57	0.89	<0.01*	0.41
p_{mol}			0.91	0.14	0.42	0.12	0.21	0.44	0.02*	0.01*	0.83	0.30	0.02*	<0.01*
$p_{treatment}$			<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*

Table S3. Mineral nutrient (N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, B and Mo) uptake in bottom leaves under the influence of low and adequate N supply, and foliar application of N and Mo. Means were compared by LSD test using p < 0.05.

N supply	Foliar treatment		N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B	Mo
	N	Mo	----------------- Botton Leaves (mg plant^{-1}) -------------------						-------------------- Botton leaves (µg plant^{-1}) ----------------					
Low	-	-	14 d	16 c	14 c	15 c	5 c	2 d	863 a	1270 c	5 c	16 d	99 b	2 b
Low	-	+	15 d	16 c	15 c	16 c	6 c	2 d	308 c	1465 bc	5 c	13 d	114 b	9 b
Low	+	-	15 d	14 c	15 c	14 c	5 c	2 d	139 fg	1262 c	4 c	12 d	100 b	2 b
Low	+	+	18 d	14 c	17 c	15 c	5 c	2 d	136 g	1490 bc	5 c	11 d	98 b	68 b
Adequate	-	-	88 a	50 a	60 a	47 a	15 a	9 a	480 b	1838 b	23 a	44 a	209 a	14 b
Adequate	-	+	48 c	37 b	39 b	33 b	11 b	6 b	218 df	1510 bc	13 b	24 c	182 a	11 b
Adequate	+	-	68 b	40 b	48 ab	36 b	15 a	8 ab	246 cd	1717 bc	17 b	29 bc	205 a	6 b
Adequate	+	+	61 b	36 b	40 b	36 b	12 ab	7 bc	227 cd	2403 a	22 a	31 b	180 a	155 a
Low			16 B	15 B	15 B	15 B	5 B	2	362 A	1372	4 b	13 B	103 B	20
Adequate			66 A	41 A	47 A	37 A	13 A	8	293 B	1867	19 a	32 A	194 A	46
	-		41	29	32	28	9	5	467 A	1521	11	25	151	9 B
	N		41	26	30	24	9	5	187 B	1718	12	21	146	58 A
		-	46 A	30	34 A	28 A	10	5	432 A	1521	12	26 A	153	6 B
		Mo	35 B	26	28 B	24 B	9	5	222 B	1717	11	20 B	143	61 A
$p_{Nsupply}$			<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	0.22
p_{N}			0.99	0.15	0.49	0.07*	0.75	0.97	<0.01*	0.13	0.79	0.08	0.52	0.03*
p_{mol}			0.02*	0.10	0.05*	0.04*	0.11	0.08*	<0.01*	0.13	0.48	<0.01*	0.23	0.01*
$p_{treatment}$			<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*

Table S4. Mineral nutrient (N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, B and Mo) uptake in stalks under the influence of low and adequate N supply, and foliar application of N and Mo. Means were compared by LSD test using $p < 0.05$.

N supply	**Foliar treatment**		**N**	**P**	**K**	**Ca**	**Mg**	**S**	**Fe**	**Mn**	**Cu**	**Zn**	**B**	**Mo**
	N	**Mol**	**Stalks (g plant^{-1})**						**Stalks (µg plant^{-1})**					
Low	-	-	28 d	29 b	106 b	22 bcd	12 b	15 b	245 c	687 c	37 c	96 c	20 b	6 c
Low	-	+	37 d	32 b	107 b	21 cd	13 b	17 b	246 c	1009 a	42 c	133 c	20 b	444 b
Low	+	-	37 d	31 b	126 b	19 d	13 b	17 b	180 c	928 ab	36 c	129 c	19 b	9 c
Low	+	+	35 d	28 b	106 b	18 d	12 b	14 b	291 bc	1009 a	31 c	121 c	18 b	348 b
Adequate	-	-	112 c	64 a	209 a	33 abcd	26 a	32 b	728 a	817 ab	90 b	212 b	54 a	5 c
Adequate	-	+	124 bc	64 a	212 a	36 abc	26 a	34 ab	588 ab	687 ab	102 ab	228 b	48 a	343 b
Adequate	+	-	142 ab	70 a	260 a	36 abc	30 a	37 ab	582 ab	723 ab	118 a	216 b	49 a	10 c
Adequate	+	+	152 a	81 a	251 a	39 a	31 a	44 a	690 a	1005 a	115 ab	290 a	50 a	636 a
Low			34 B	30 B	111 B	20 B	13 B	16 B	241	964	37 B	120 B	19 B	202
Adequate			132 A	70 A	233 A	36 A	28 A	37 ab	647	808	106 A	236 A	50 A	249
	-		75	47	159	28	19	24	452	856	68	167	35	199
	N		92	53	186	28	22	28	436	916	75	189	34	251
		-	80	49	175	27	20	25	434	844	70	163	36	8 B
		Mo	87	51	169	29	20	27	454	927	73	193	34	443 A
$p_{Nsupply}$			<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	0.19
p_{N}			<0.01*	0.22	0.14	0.98	0.27	0.18	0.83	0.40	0.29	0.06	0.82	0.15
p_{mol}			0.25	0.52	0.73	0.75	0.99	0.39	0.79	0.25	0.74	0.01*	0.74	<0.01*
$p_{treatment}$			<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*

Table S5. Mineral nutrient (N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, B and Mo) uptake in roots leaves under the influence of low and adequate N supply, and foliar application of N and Mo. Means were compared by LSD test using $p < 0.05$.

N supply	**Foliar treatment**		**N**	**P**	**K**	**Ca**	**Mg**	**S**	**Fe**	**Mn**	**Cu**	**Zn**	**B**	**Mo**
	N	**Mo**	**---------------------- Roots (g plant^{-1}) -------------------------**						**--------------------------- Roots (µg plant^{-1})-------------------------**					
Low	-	-	35 c	26 b	95 c	36 bc	26 c	22 c	1327 b	1578 abc	22 c	66 d	33 c	2 d
Low	-	+	42 c	29 b	91 c	31 c	30 c	29 c	1571 b	1920 a	29 bc	74 d	38 c	87 ab
Low	+	-	45 c	28 b	89 c	37 bc	30 c	26 c	1500 b	1735 ab	22 c	76 d	35 c	1 d
Low	+	+	44 c	30 b	77 c	34 bc	29 c	30 c	1681 b	1859 a	35 bc	86 d	37 c	132 a
Adequate	-	-	101 b	62 a	240 ab	46 ab	50 b	77 b	1954 ab	1105 c	39 bc	135 c	55 b	21 cd
Adequate	-	+	114 ab	65 a	276 a	54 a	55 ab	77 b	2924 a	1256 bc	67 a	152 bc	75 a	40 bcd
Adequate	+	-	121 a	67 a	286 a	61 a	60 a	94 a	1748 b	1266 bc	49 ab	167 ab	90 a	2 d
Adequate	+	+	108 ab	61 a	203 b	47 ab	51 ab	77 b	2165 ab	1538 abc	63 a	184 a	78 a	63 bc
Low			41 B	28 B	88 B	34 B	29 B	27 B	1529 B	1773 A	27 B	75 B	36 B	56
Adequate			111 A	64 A	251 A	52 A	54 A	81 A	2198 A	1291 B	55 A	160 A	75 A	42
	-		73	46	175	42	40	51	1944	1465	40	107	50 B	38
	N		80	46	164	45	43	57	1774	1599	42	128	60 A	50
		-	75	46	177	45	42	55	1632	1421 B	33 B	111	53	7 B
		Mo	77	46	162	41	41	53	2085	1643 A	49 A	124	57	81 A
$p_{Nsupply}$			<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	0.13
p_{N}			0.11	0.75	0.43	0.44	0.34	0.11	0.48	0.25	0.61	<0.01*	0.03*	0.44
p_{mol}			0.70	0.91	0.29	0.31	0.78	0.64	0.07	0.06*	<0.01*	0.09	0.41	<0.01*
$p_{treatment}$			<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*

Table S6. Mineral nutrient (N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, B and Mo) uptake in total uptake leaves under the influence of low and adequate N supply, and foliar application of N and Mo. Means were compared by LSD test using $p < 0.05$.

N supply	Foliar treatment		N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B	Mo
	N	Mo	Total uptake (mg plant^{-1})						Total uptake (µg plant^{-1})					
Low	-	-	92 d	69 b	227 b	72	44 c	39 c	1853 c	3196 d	67 c	184 c	102 d	22 d
Low	-	+	111 d	77 b	229 b	68	49 c	49 c	1988 bc	3801 bcd	80 c	232 c	123 cd	935 c
Low	+	-	118 d	73 b	241 b	70	48 c	46 c	1797 c	3464 cd	67 c	233 c	122 cd	23 d
Low	+	+	117 d	72 b	212 b	66	45 c	47 c	2834 abc	3816 bcd	75 c	229 c	173 c	933 c
Adequate	-	-	392 bc	204 a	560 a	144	99 b	125 b	3381 ab	4207 bc	175 b	459 b	376 b	47 d
Adequate	-	+	379 c	209 a	607 a	164	104 ab	127 b	4028 a	4322 bc	209 ab	469 b	422 ab	1371 b
Adequate	+	-	440 a	214 a	681 a	172	117 a	149 a	2863 abc	4607 b	212 ab	482 b	475 a	62 d
Adequate	+	+	419 ab	210 a	563 a	152	106 ab	138 ab	3322 ab	5830 a	224 a	561 a	413 b	2039 a
Low			109 B	73 B	228 B	69 B	47 B	45 B	2117 B	3569 B	72 B	220 B	130 B	478 B
Adequate			408 A	409 A	410 A	158 A	107 A	135 A	3398 A	4741 A	205 A	493 A	421 A	880 A
	-		243 B	140	406	112	74	85 B	2812	3881 B	133	336 B	256	594
	N		274 A	142	424	115	79	95 A	2704	4429 A	144	377 A	296	765
		-	260	140	427	114	77	90	2473	3868 B	130	340 B	269 B	39 B
		Mo	257	142	403	112	76	90	3043	4441 A	147	373 A	283 A	1320 A
$p_{Nsupply}$			<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*
$p_{Nfoliar}$			<0.01*	0,61	0,53	0,71	0,24	0.05*	0,73	0.02*	0,31	0.02*	0,35	0,09
p_{mol}			0,63	0,71	0,42	0,79	0,81	0,92	0,09	0.02*	0,14	0.04*	0.03*	<0.01
$p_{treatment}$			<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*

Table S7. Nitrogen derived from fertilizer (NDFF) and [15]N-fertilizer Recovery under the influence of low and adequate N supply, and foliar application of N and Mo. Means were compared by LSD test using $p < 0.05$.

N supply	Foliar treatment		Top leaves	Botton Leaves	Stalk	Roots	Top leaves	Botton Leaves	Stalk	Roots	Total
	N	Mo	Nddf (%)				Recovery (%)				
Low	-	-	0 c	0 c	0 c	0 c	0 c	0 b	0 b	0 c	0 b
Low	-	+	0 c	0 c	0 c	0 c	0 c	0 b	0 b	0 c	0 b
Low	+	-	24 a	10 a	18 a	9 a	11 b	3 a	11 a	6 ab	30 a
Low	+	+	23 a	12 a	17 a	12 a	11 b	3 a	9 a	6 a	29 a
Adequate	-	-	0 c	0 c	0 c	0 c	0 c	0 b	0 a	0 c	0 b
Adequate	-	+	0 c	0 c	0 c	0 c	0 c	0 b	0 a	0 c	0 b
Adequate	+	-	12 b	2 b	6 b	3 b	17 ab	3 a	11 a	4 b	35 a
Adequate	+	+	14 b	3 b	5 b	2 b	21 a	2 a	11 a	3 b	37 a
Low			12 A	6 A	9 A	6 A	6 B	1	5	3	15
Adequate			7 B	1 B	3 B	1 B	10 A	1	5	2	18
	-		0 B	0 B	0 B	0 B	0 B	0 B	0 B	0 B	0 B
	Nfol		18 A	7 A	12 A	6 A	15 A	3 A	10 A	5 A	33 A
		-	9	3	6	3	7	1	5	3	17
		Mo	10	4	6	3	8	1	5	2	17
$p_{Nsupply}$			<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	0,61	0,68	0,10	0,17
$p_{Nfoliar}$			<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*
p_{mol}			0,52	0,76	0,81	0,87	0,41	0,79	0,68	0,73	0,76
$p_{treatment}$			<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*

Table S8. N-NO_3 and N-NH_3 (g kg^{-1}) in the plant compartments under the influence of low and adequate N supply, and foliar application of N and Mo. Means were compared by LSD test using $p < 0.05$.

N supply	**Foliar treatment**		**Top leaves**		**Botton Leaves**		**Stalks**		**Roots**	
	N	**Mo**	**N-NO_3**	**N-NH_3**	**N-NO_3**	**N-NH_3**	**N-NO_3**	**N-NH_3**	**N-NO_3**	**N-NH_3**
Low	-	-	0.02 c	0.56 d	0.02 cd	0.47 cd	0.03 c	0.25 c	0.04 c	0.34 c
Low	-	+	0.02 c	0.66 bcd	0.01 d	0.67 bc	0.03 c	0.31 c	0.05 c	0.43 bc
Low	+	-	0.05 ab	0.71 abc	0.01 d	0.46 cd	0.03 c	0.29 c	0.07 c	0.91 a
Low	+	+	0.04 ab	0.76 ab	0.03 bc	0.67 bc	0.04 c	0.51 b	0.10 bc	0.87 a
Adequate	-	-	0.05 a	0.6 cd	0.05 a	0.79 b	0.07 a	0.75 a	0.21 a	0.37 bc
Adequate	-	+	0.02 c	0.62 cd	0.04 ab	0.49 cd	0.04 bc	0.53 b	0.18 ab	0.52 b
Adequate	+	-	0.04 ab	0.65 cd	0.01 d	1.13 a	0.04 bc	0.69 a	0.13 abc	0.49 bc
Adequate	+	+	0.03 bc	0.82 a	0.01 d	0.34 d	0.06 ab	0.5 b	0.13 abc	0.44 bc
Low			0,03	0,67	0,02	0,57	0.03 B	0.34 B	0.07 B	0.64 A
Adequate			0,04	0,67	0,03	0,69	0.05 A	0.62 A	0.16 A	0.45 B
	-		0.03 B	0.61 B	0.03 A	0,63	0,04	0,46	0,12	0.42 B
	Nfol		0.04 A	0.74 A	0.01 B	0,65	0,04	0,50	0,11	0.67 A
		-	0.04 A	0.63 B	0,02	0,71	0,04	0,49	0,11	0,53
		Mo	0.03 B	0.72 A	0,02	0,57	0,04	0,46	0,12	0,56
$p_{Nsupply}$			0,23	0,53	0,07	0,25	<0.01*	<0.01*	<0.01*	<0.01*
$p_{Nfoliar}$			<0.01*	<0.01*	0.03*	0,65	0,67	0,46	0,70	<0.01*
p_{mol}			0,02	<0.01	0,81	0,11	0,73	0,53	0,89	0,57
$p_{treatment}$			<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*

FOLIAR SPRAY WITH NITROGEN AND MOLYBDENUM IN SUGARCANE UNDER FIELD CONDITIONS

Abstract

The proper nitrogen (N) management in sugarcane plays a major role in productivity and low carbon emissions. A limited response of green harvested sugarcane to N applied to the soil, documented in recent studies, opens an opportunity to reduce the N rates applied to the soil by combining with foliar spray of N. In this sense, the objective of this study was to determine how foliar applications of N rates combined with molybdenum (Mo) affected the production and quality of sugarcane. The study was carried out in nine fields of ratoon sugarcane stage, under green harvested fields, during two crop seasons (2018/2019 and 2019/2020). Nitrogen was applied at a rate of 0.8 kg N t^{-1} of stalk harvested, in addition to 50 kg ha^{-1} P_2O_5 and 140 kg ha^{-1} K_2O. Foliar applications were carried out during the period of maximum vegetative growth (i.e., from November to March) and the experimental design was a factorial 4 x 2 + 1, including four N rates (control; 5; 10 and 20 kg ha^{-1} N as urea), with and without Mo supply (100 g ha^{-1}), plus an additional control without N application to the soil. As a result, foliar application increased leaf Mo content by up to 167%, but no changes were observed in foliar N content. In addition, Mo application reduced productivity and increased ATR in the first year of the study. A significant drought period following N and Mo application in the first crop season (2018/2019) caused reduction in yields when N rates of 10 and 20 kg ha^{-1} N In the crop season 2019/2020, under normal rainfall distribution, the rate of 5 kg ha^{-1} N increased sugarcane yield by 6 Mg ha^{-1} in the average of nine fields, whereas N rates of 10 and 20 kg ha^{-1} N yielded similar to control treatment. . Although Mo did not influence the average yield of the second year of study, it increased sugar concentration in the stalks. Results of this study demonstrate the possibility of combining 0.8 kg N t^{-1} of stalk in soil application plus 5 kg ha^{-1} N in foliar spray, whereas positive effects of foliar spray depend on adequate rainfall distribution.

Key words: Foliar fertilization, nitrogen spray, foliar molybdenum.

3.1 Introduction

Sugarcane nutrition is required for the crop to express its maximum production potential in an environmentally sustainable and profitable way. Therefore, the role of nitrogen (N) can be highlighted, which presents a complex dynamic, due to the multiple transformations and its mobility in the soil-plant system. In this context, the N management fertilization stands out as one of the most studied cultural practices in the sugarcane culture, thus existing a great demand for research (Cantarella et al., 2007).

Nitrogen fertilization is important for the carbon balance of sugarcane ethanol production, resulting in a great demand for research aiming to improve the N use efficiency (NUE) of the crop. Inadequate management of N fertilization can reduce the mitigation of CO_2 emissions by 30% from the replacement of fossil fuels with sugarcane ethanol (Jaiswal et al., 2017). The carbon balance advantage of biofuels compared to fossil fuels can be offset depending on the amount of N_2O emitted during biofuels-crop cultivation (Crutzen et al., 2008).

The main causes of low NUE is the lack of synchronism between the N demand and fertilization, asymbiotic N fixation, as well as N applications not considering the N provided by the mineralization of soil organic matter (Cantarella et al., 2007).

Several strategies can be used to increase NUE in agricultural crops. The most promising are adjusting fertilizer-N rates (Otto et al., 2019; Otto et al., 2020; Castro et. al. 2021), split N application (Otto et al., 2020), leaf N application (Castro, 2022), the use of controlled release fertilizers (Villalba et al., 2014; Guelfi, 2017), fertilizer incorporation into the soil (Castro et al., 2016) and urease and nitrification inhibitors usage (Cantarella, 2007; Silva et al., 2017).

Adjusting the N rates is the main and most tested way to increase the NUE in sugarcane. Several studies have shown, in most cases, increasing N rates does not necessarily increases sugarcane yield (Otto et al., 2016; Contin, 2007). In a literature review with green harvested sugarcane, Otto et al. (2016), surveyed the response to N fertilization in 45 experiments and found that 75% of the areas are moderate or unresponsive to N. Therefore, in most cases, increasing the N rates does not necessarily increase yields (Contin, 2007; Otto et al., 2020).

The other strategies have been exhaustively studied, such as the use of urease and/or nitrification inhibitors (Costa et al., 2003; Cantarella et al., 2008; Mariano et al., 2012; Mira et al., 2017), location and incorporation of fertilizer (Vitti et al., 2007; Castro et al., 2016; Silva et al., 2017), variation of sources of N (Costa et al. 2003; Vitti et al., 2007; Boschiero, 2017), and N application at varying rates (Amaral et al., 2015). More recently, Otto et al. (2020) found that only seven of the 25 site-years were responsive to N fertilization in the ratoon stage, and identified, on the average of the responsive sites, that sugarcane yields were similar to N rates of 0.8, 1.0, 1.2, and 1.4 kg N t^{-1} of stalks. This represents an advancement for soil N application in sugarcane fields, since most growers used, at the time of the study, 1.2 kg N t-1 of stalks. Results of that study demonstrate an opportunity to growers to reduce the N rates applied to the soil, further complementing an extra amount of N in the maximum vegetative growth stage to maximize yields.

The efficiency of foliar N fertilization depends on several factors, including the plant nutritional status, stage of crop development, leaf age, leaf surface properties and climatic conditions at the time of application (Fageria et al., 2009; Fernandez and Eicherr, 2009).

Although the effect of foliar N application on the different nutritional plant status has been investigated, the results remain unclear. Some studies on hydrangea, apple and citrus point to greater absorption and translocation of N-urea leaf applied in plants with low foliar N content (Cheng et al., 2002; Bi and Scagel, 2008; Lea-Cox and Syvertsen, 1995). On the other hand, some authors demonstrate that foliar N absorption is lower in plants with nutrient deficiency due to a higher concentration of cuticular wax (Bondada et al. 2001; Klein and Weinbaum, 1984).

However, there is no study on the effect of leaf N application on micronutrient deficient plants. The assimilation of N in organic compounds depends on a sequence of reactions in which nitrate reductase (soluble molybdoflavoprotein) participates, an enzyme that has Mo in its composition (Campbell, 1999). A series of experiments carried out recently indicated a significant sugarcane response to the application of micronutrients in the planting furrow (Mellis, et al., 2016). The authors observed that Zn and Mo were the micronutrients that showed the most significant effects, with gains of 19 and 12 Mg ha^{-1} sugarcane stalk yield (SSY), respectively, in the average of eleven experiments. It demonstrates the need of reliable methods to apply micronutrients for sugarcane fields, in order to achieve maximum yields.

Associated with N fertilization, the foliar fertilizer industry launched a range of products with micronutrients and compounds such as synthetic hormones, amino acids, humic and fulvic acids and seaweed extracts on the market. Despite the great availability of those products in the market, scientific validation is still missing. Among the micronutrients that accompany N foliar fertilization, molybdenum (Mo) stands out, a nutrient with a direct participation in N metabolism (Wei, Li and Yang, 2007; Santos, 2014; Thapa, Prasad and Rai, 2016; Silva et al., 2016).

In this study, we hypothesized that the application of moderates N rates to the soil (0.8 kg N t-1), in association with foliar application of N and Mo, in the maximum growth stage, will result in maximum sugarcane yield and NUE. Thus, the objective of this study was to evaluate N rates foliar spray and the interaction with the use of Mo on the yield and the crop nutritional status, under different locations of green harvested sugarcane cultivation in Brazil.

3.2 Material and Methods

3.2.1 Sites characterization

Five experiments were conducted in commercial sugarcane fields harvested without burning in the south-central region of Brazil in the municipalities of Ivinhema – MS, Ilha

Solteira – SP, São Pedro do Turvo – SP, Onda Verde – SP, Suzanapolis – SP (Figure 1). The trials were established in sugarcane first ratoons in the middle of the 2017/2018 season. Fertilizers were applied to the soil up to 90 days after the harvest, and the foliar applications were carried out in the summer period, in the maximum growth stage of sugarcane crop, when the crop reached the inter-row closure. At all sites, the same plots were reinstalled and conducted in the 2018/2019 season (Table 1).

According to the Köppen classification, the field climate was classified as Aw (savanna) in all trials except São Pedro do Turvo-SP, which was classified as Am (monsoon). Weather data were obtained from meteorological stations near the sites.

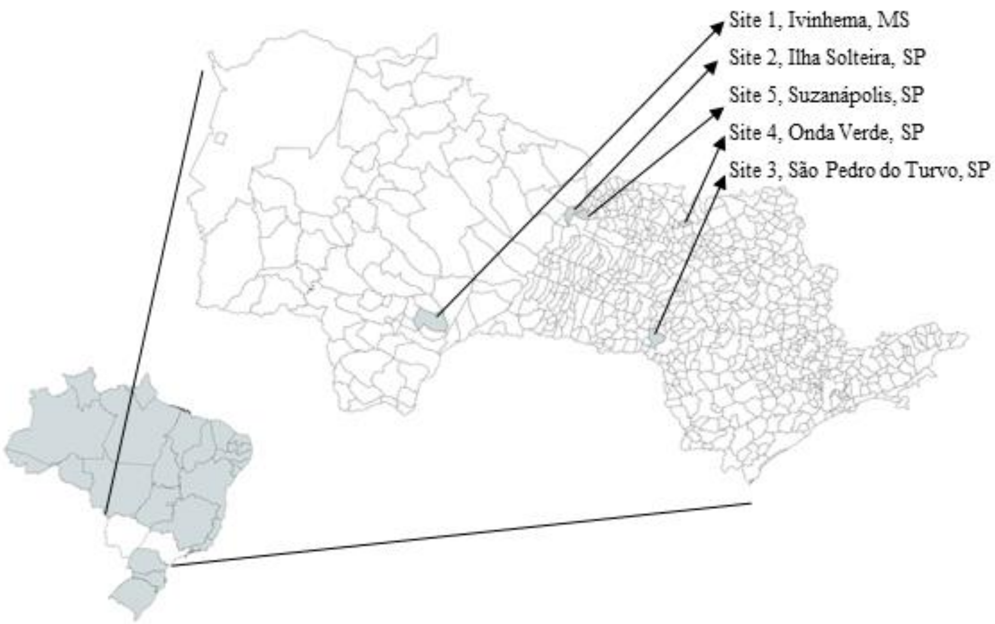


Figure 1. Experimental sites located at Ivinhema-MS (Site 1, Adecoagro Group; 22.305544, -53.960433), Ilha Solteira-SP (Site 2, Raízen Group; -20.480442, -51.112258), São Pedro do Turvo-SP (Site 3, São Luiz Mill; -22.813923, -49.818891), Onda Verde-SP (Site 4, Tereos Group; -20.682171, -49180535), Suzanápolis-SP (Site 5, Vale do Paraná Mill; -20.643517, -51.317750)

There was an intense drought period throughout in the south-central region of Brazil following the application in the 2017/2018 crop season. In the second crop season (2018/2019), the applications were carried out at the beginning of January and there were no drought period following applications. Rainfall during the 2 months preceding the foliar application was 47% lower on average in the 2017/2018 crop season compared to the 2018/2019 crop season (Figure 2).

The soil was sampled immediately before the trials were set up for physical (Camargo at al., 2009) and chemical characterization (Raij, Andrade and Cantarella, 2001). Soil samples were collected from ten locations at 0.25 m depth intervals up to 1.0 m. Samples were taken at 0.25 m apart from the sugarcane row and the results presented in Table 2. Soil classification was performed according to the Soil Survey Staff (2022).

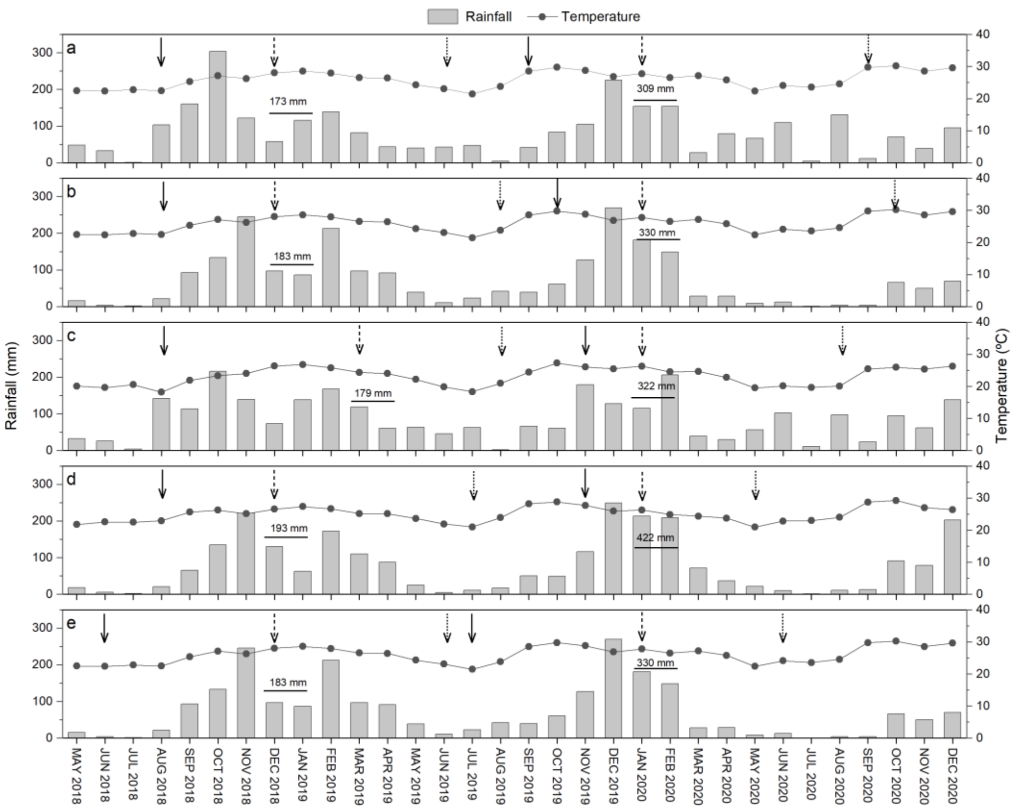


Figure 2. Rainfall (mm) and Temperature (ºC) of the sites during 2018 and 2020. The arrows with solid line represent time of soil fertilization; Dash line: foliar fertilization; Dot line: harvest.

The soil was prepared using the conventional system during the establishment of all sites, which included plowing, disking, harrowing, and furrowing. Before planting, lime and gypsum were applied at rates ranging from 1.0-4.0 and 0.8-1.5 Mg ha^{-1}, respectively, to achieve 70% base saturation at a soil depth of 0.25 m. Fertilization was done in the furrow with potassium (K) and phosphorus (P) according to the recommendations of Raij et al. (1997). We certified no vinasse or organic residues had been used in previous year prior to the trial’s establishment because these practices reduce the sugarcane N response (Otto et al., 2013).

Table 1. Soil type, sugarcane cultivar, date of establishment, date of foliar application and harvest in each season.

Site	Variety	Soil type	2018/2019 season			2019/2020 season		
			Establish	Foliar application	Harvest	Establish	Foliar application	Harvest
1	RB966928	Typic Eutrudox	Aug/18	Dec/18	Jun/19	Set/19	Jan/20	Jul/20
2	CTC9001	Typic Quatzipsamment	Aug/18	Dec/18	Aug/19	Oct/19	Jan/20	Oct/20
3	CTC4	Typic Hapludox	Aug/18	Mar/19	Aug/19	Nov/19	Jan/20	Set/20
4	RB966928	Typic Hapludox	Aug/18	Dec/18	Jul/19	Nov/19	Jan/20	Jun/20
5	RB92579	Typic Eutrudox	Jun/18	Dec/18	Jun/19	Jul/19	Jan/20	Jun/20

Table 2. Soil chemical and physical properties at each site.

Site	Soil depth	pH	O.M.	P	S	K	Ca	Mg	Al	H+Al	SB	CEC	V	Al	Sand	Silt	Clay
	cm		g dm^{-3}	mg dm^{-3}		---------------------- mmolc dm^{-3} -----------------							------- % -------		-------- g kg^{-1} --------		
1	0-25	5.1	11	12	9	<0.9	34	3	<0.02	11	37.8	48.8	77	0	766	47	187
	25-50	5.3	9	14	12	<0.9	28	2	1	11	30.5	41.5	73	3	788	38	174
	50-75	4.5	<5	9	25	<0.9	23	2	6	22	25.3	47.3	53	19	724	31	245
	75-100	4.3	<5	8	9	<0.9	23	1	7	18	24.5	42.5	58	22	735	12	253
2	0-25	5.8	12	10	<6	<0.9	30	3	1	9	33.4	42.4	79	3	917	12	76
	25-50	4.8	10	8	<6	<0.9	5	2	1	9	7.5	16.5	45	12	900	25	75
	50-75	4.9	7	10	<6	<0.9	10	3	<0.02	9	13.6	22.6	60	0	869	31	100
	75-100	4.8	<5	17	<6	<0.9	6	2	1	9	8.6	17.6	49	10	829	35	126
3	0-25	5.8	25	16	<6	0.9	58	20	<0.02	28	78.9	106.9	74	0	318	124	558
	25-50	5.3	18	8	10	<0.9	28	9	2	47	37.4	84.4	44	5	302	116	582
	50-75	4.9	15	5	19	<0.9	11	5	3	58	16.4	74.3	22	16	273	119	608
	75-100	4.7	14	4	38	<0.9	9	4	4	58	13.2	71.2	19	23	249	143	608
4	0-25	5.2	12	6	8	1.6	11	4	<0.02	12	16.6	28.6	58	0	765	11	225
	25-50	5.1	8	5	15	<0.9	6	3	1	12	9.7	21.7	45	9	696	30	273
	50-75	5.1	<5	<3	63	<0.9	5	2	1	12	7.5	19.5	38	12	743	21	236
	75-100	5.2	<5	<3	92	<0.9	5	3	<0.02	12	8.6	20.6	42	0	674	27	299
5	0-25	5	13	11	<6	1	29	5	<0.02	10	35	45	78	0	808	65	126
	25-50	5.4	6	8	8	0.9	25	4	<0.02	10	29.9	39.9	75	0	734	89	177
	50-75	5.3	<5	8	8	1	30	5	<0.02	10	36	46	78	0	703	70	227
	75-100	5.3	<5	7	7	1	29	4	<0.02	9	34	43	79	0	694	80	226

pH in 0.01 mol^{-1} CaCl2; SB: sum of bases; CEC, cation exchange capacity; V%, bases saturation; Al%, aluminum saturation

3.2.2 Experimental design

The experiment was designed as a factorial 2x4+1, with and without application of Mo (100 g ha^{-1} Mo), four rates of N (control, 5, 10 and 20 kg ha^{-1} N), plus an additional control without application of N in the soil. Experimental units were designed with six sugarcane rows with a length of 15 m, spaced 1.5 m apart and the plot size was 135 m^2 (9 × 15 m).
Previous to foliar application, N fertilizer was applied in band, 0.2 m from the row, with no incorporation of ammonium nitrate (33% N), which has negligible ammonia losses after surface application in acidic soils (Figure 4A, supplementary). We choose the rate of N for soil application of 0.8 kg N Mg^{-1} stalk based on the previous study of Otto et al. (2021). To avoid nutritional deficiencies, 50 kg ha^{-1} P_2O_5 (triple superphosphate) and 150 kg ha^{-1} K_2O (potassium chloride) were also applied, following the recommended nutritional practices (van Raij et al., 1997). The varieties were chosen based on sugarcane mill guidelines to match the best maturation period and soil conditions at each site. All cultivars were representative of south-central cultivation practices. Foliar fertilizers were applied during the period of highest vegetative growth (December to March), using a pressurized CO_2 pump (1.5 bar), at a rate flow of 150 L ha^{-1}. The N source used was urea (45% of N) at the rates of 0, 5, 10 and 20 kg ha^{-1} of N, and sodium molybdate (39% of Mo) at the rate of 100 g ha^{-1} of Mo. The applications were carried out in the early morning under conditions of relative humidity greater than 80% and temperature lower than 30°C (Figure 4B, supplementary).

3.2.3 Sugarcane measurements and analysis

After 40 days of foliar fertilizer was applied, 20 leaf samples were collected per plot, collecting the top visible dewlap (TVD) leaf - the first leaf with fully expanded auricles (leaf +1) (Figure 4C, supplementary). Only the third part of the plant, excluded the central vein, was used for analysis, following the guidelines of Raij et al (1997). Before leaf sampling, in the middle third of the leaves, the SPAD index was also evaluated, which indirectly estimates the chlorophyll content of the leaves. Foliar samples were washed as follows: washing with distilled water with 1 ml l^{-1} of neutral detergent in the first tray, bathing in solution with 0.1 M l^{-1} of HCl in the second tray, and rinse in distilled water. Then the leaves were dried in a stove at 50°C for 72 hours, ground in a Wiley-type mill and submitted to tissue analysis according to the methodology described by Malavolta, Vitti and Oliveira (1997).

Stalk yield was calculated by harvesting the four central rows (90 m^2) of each plot mechanically, in both years of study. The harvested stalks were weighed in an automated truck equipped with a cell loading system, and sugarcane yield (Mg ha^{-1}, fresh basis) was estimated.

The diameter and height of the stalks were measured immediately before harvest, and 10 stalks per plot were randomly collected to determine theoretical recoverable sugar (TRS; kg sugar Mg^{-1} of stalk) (Fernandes et al., 2003). Sugar yield (Mg ha^{-1}) was calculated using sugarcane sugar concentration and stalk yield.

3.2.4 Statistical analysis

Data exploration was performed using principal component analysis (PCA) to identify patterns and relationships among the foliar nitrogen and molybdenum whit the yield parameters and nutritional status. The Shapiro-Wilk and Levene tests ($P>0.1$) were used to assess the assumptions of normality and variance homogeneity. When Grubbs' test detected outliers, they were removed. The F-test was used to submit data from each harvest to ANOVA. When the F-test was significant ($P<0.1$), the LSD test was used to assess the effect of Mo and N rate ($P< 0.1$). The treatment control (i.e., without soil N fertilization), was evaluated by t-test contrast ($P< 0.1$) against the treatment with N in the soil without receiving foliar application.

3.3 Results

3.3.1 Exploratory analysis

The PCA data (Figure 3), demonstrate a significant proportion of the variation in the first year of study data by the first two principal components responsible for 23.9% and 29.4% of the data, respectively. Also, the PCA was able to separate the sites with the components. The high yield of site 5 (116.8 Mg ha^{-1} SSY) grouped the site on the yield axis represented by the PCA2, which has positively correlated with leaf N and Ca. On the other hand, Site 2, had the highest leaf content of K (16.5 g kg^{-1} K), Cu (20.5 mg kg^{-1} Cu), Zn (5.2 mg ka^{-1} Zn), and Mn (125 mg kg^{-1} Mn), and had the lowest yield (82.3 Mg ha^{-1} SSY) (Figure 3).

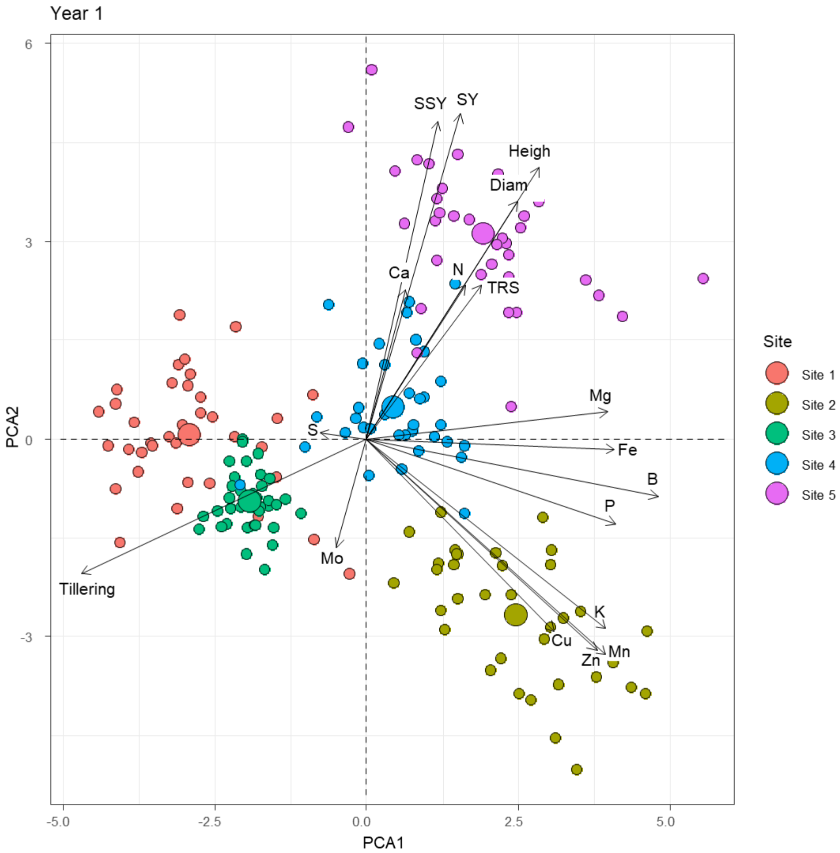


Figure 3. Principal component analysis of the first crop season, based on the variables: sugarcane stalk yield (YYD), sugar yield (SY), theoretical recoverable sugar (TRS), heigh and diameter of stalks, tillering, N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, B and Mo foliar content. The 2 components represent 53.3% of the parameter's variability.

In the second year, PCA analysis was able to predict 61.6% of the data variation with the 44.5% and 17.1% at the first and second dimensions, respectively. Site 5 pulled the yield axis (104.5 Mg ha^{-1} SSY) and site 2 pulled the negative correlation of Mn, Cu and Zn concentration with productivity. The highest positive correlations with productivity were Ca and S. Site 3 had the highest levels of Ca, Mg, and Fe (Figure 4).

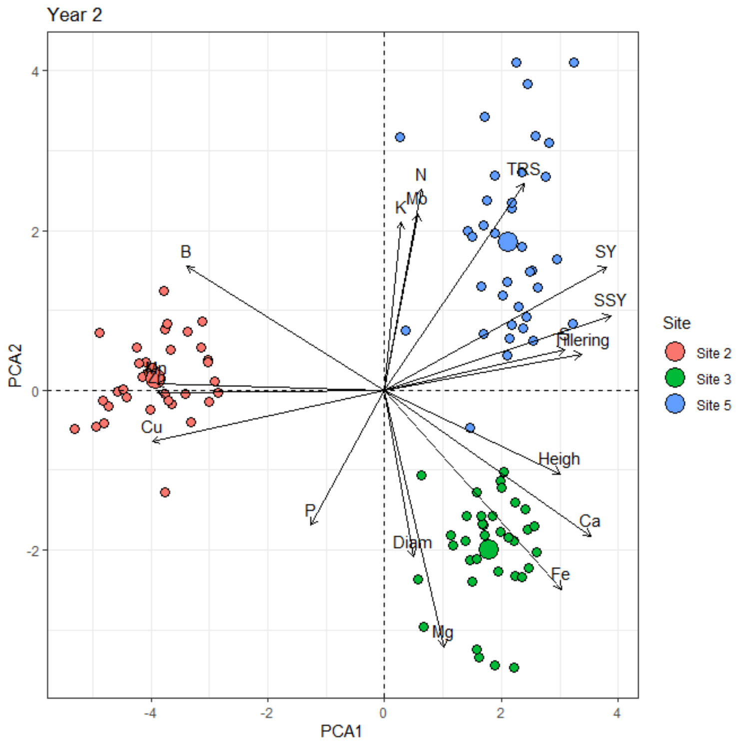


Figure 4. Principal component analysis of second crop season, based on the variables: sugarcane stalk yield (YYD), sugar yield (SY), theoretical recoverable sugar (TRS), heigh and diameter of stalks, tillering, N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, B and Mo foliar content. The 2 components represent 61.6% of the parameter's variability.

3.3.2 Foliar contents of minerals

In the average of the two years, there was no increase in leaf N content with the application of N foliar rates. Leaf N content was also not influenced by the Mo application. In the first year, there was a negative effect of the highest rate (i.e., 20 kg ha^{-1} N) on the N content in the plot without Mo application (Figure 5). Also, the same negative effect can be noticed at site 4 during the first crop season (Table S7). There was an increase in N content only in the second year in area 5, where rates 5, 10 and 20 presented 1 g kg^{-1} more than control plots on average (Table S7). This increment of N in the leaf was converted into yield up to 17 Mg ha^{-1} SSY more in the treatment with 5 kg ha^{-1} of N compared to the control (Table 4).

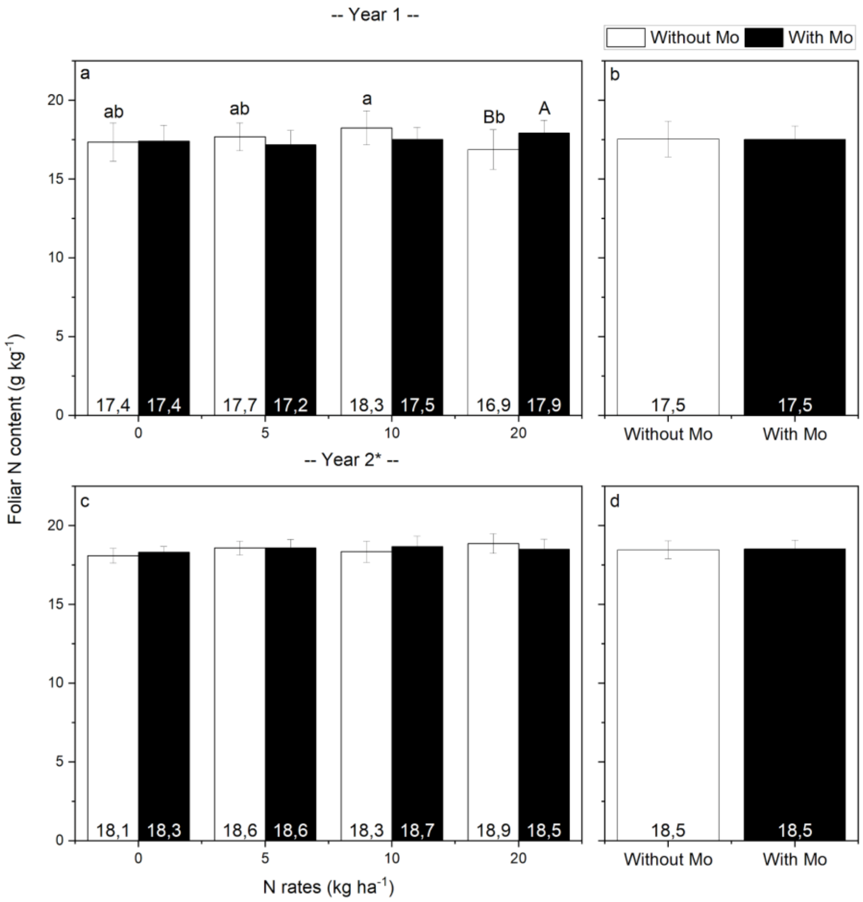


Figure 5. N foliar content in (a)Average of sites for N and Mo interaction in the Year 1, (b) Average of sites for Mo effect in the Year 1, (c) Average of sites for N and Mo interactions in the Year 2, (d) Average of sites for Mo effect in the Year 2. Means were compared by LSD teste using $p < 0.1$. Lowercase letters compared N rates and uppercase letters compare Mo effect.

There was an increase in leaf Mo content in all locations and on average across the two crop seasons. The average increment of Mo was 60 to 150% and 80 to 167% in the first and second season, respectively (Figure 6).

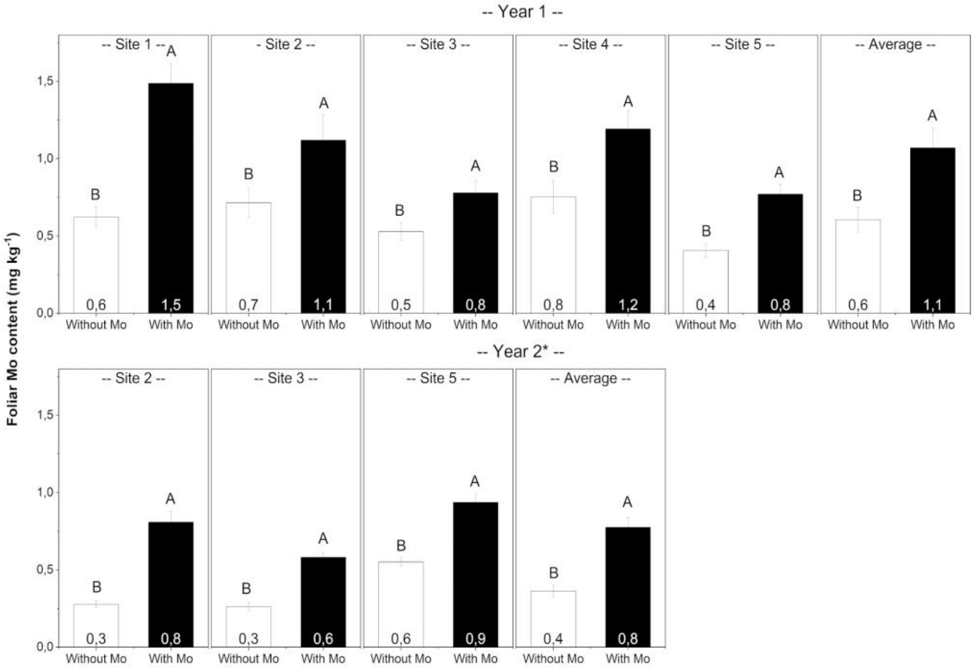


Figure 6. Leaf Mo content in the treatments with or without Mo foliar spray. Due to operational limitations, sites 1 and 4 were not sampled in the second year of conducting the test. Means were compared by LSD teste using $p < 0.1$.

Without the Mo application, the levels of P decreased with the 10 and 20 kg ha^{-1} of N rates in site 1 in the first year. Site 5 showed the same result regardless of the application of Mo, the P content decreased 18% in the 10 and 20 kg ha^{-1} N rates. It was also observed 14% less P foliar content when Mo was not applied in site 5, in the second year (Table S8). The N rates of 10 and 20 kg ha^{-1} also decrease 6% of K foliar content in the average of the first year compared to the plot without N foliar application (Table S9)

The N foliar application reduced Ca contents when Mo was not used in site 1, in the first year. The use of Mo increased Ca contents in site 3 in the first year, and in site 2 in the second year, indicating an improvement in Ca nutrition with the application of Mo associated with better yield with the use of Mo in those sites (Table S10).

The Sulphur (S) foliar content showed interaction of N and Mo in site 2 and 5 in the first year and site 5 in the second year. The interactions favored higher S contents in the plots that received higher N rates with the Mo application. In site 5 in the first year, the application of 10 and 20 kg ha^{-1} N without Mo, reduced the S levels in the leaf up to 40% (Table S12).

The N application decreases the iron (Fe) foliar contents in the site 5 and in the sites average, rates of 10 and 20 kg ha^{-1} N decrease de Fe foliar contents. In the second year, the application of 20 kg ha^{-1} N with Mo, increase the Fe foliar content (Table S13). In the average of the first year, the zinc (Zn) contents decreased with the application of foliar N up to a 7% lower at the rate of 20 kg ha^{-1} N compared to the control (Table S15). There was an interaction of N and Mo in the leaf contents of Cu in site 5 in both years.

The Cu content was lower with 20 kg ha^{-1} N without Mo and lower with 10 kg ha^{-1} N with Mo in the first and the second year, respectively. In site 3, in the first year, any N rate increased the Cu content compared to the N control plots (Table S16). In site 3 (first year) and in site 2 (second year), the N rates generated an increase in leaf Cu content (Table S16).

Boron (B) leaf contents were not influenced by foliar N application. In the first year in site 4, the Mo application reduced the B concentration by 11%. However, in the second year, the Mo application increased the B concentration by 169% and 100% in sites 3 and 5, respectively. Overall, the N rates in minerals leaf content elicited negative significant responses. In other words, the highest rates of N decreased the concentration of other nutrients including P, K, Ca, S, Mg and Zn. In addition, the application of Mo was highlighted in the increase in the concentration of Ca and B.

3.3.3 Response to N soil fertilization

The response to N fertilization in the soil was evaluated through a contrast analysis (t-test, $p < 0.1$) between the control (plot without N in the soil) and the plot with N in the soil and without application of foliar fertilization. Due to the low degree of freedom of this analysis for the isolated sites, the analysis of the average of the sites becomes important for this evaluation (Figure 7).

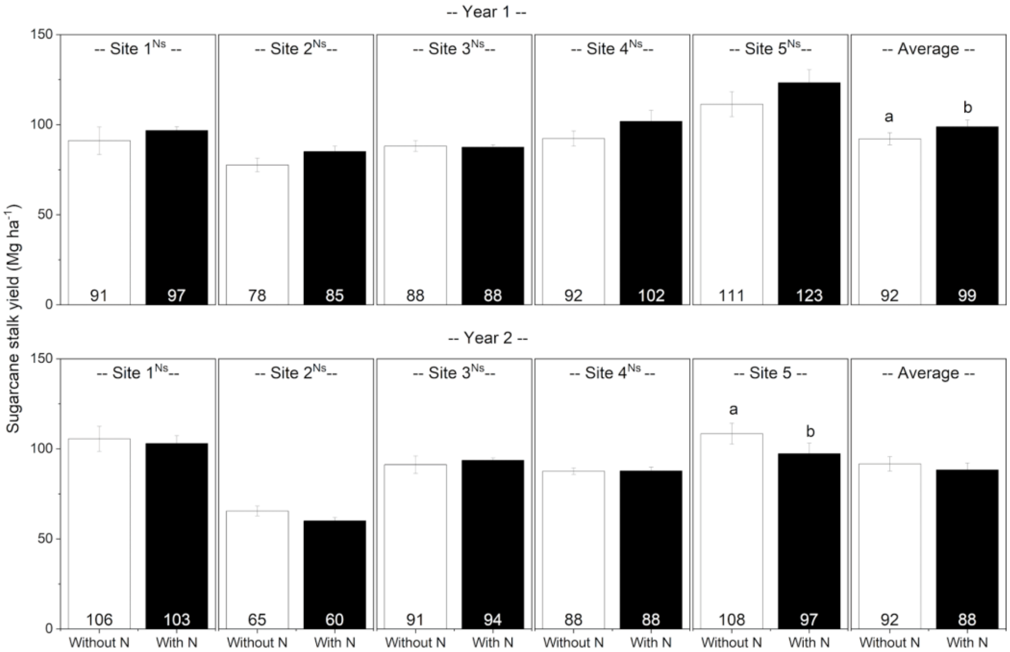


Figure 7. Response of N fertilization in the soil in the 5 sites and in the average of the sites, in the first and second year. Contrast analysis between the control treatment without application of N in the soil against the treatment that received N in the soil and did not receive foliar application. Average was compared by the t-test using p<0.1.

In year 1, site 1 stalks were 8% taller with N fertilization and the yield was 6% higher (Figure 7). In this site, an increase of 21% in the SPAD index and a decrease of 14% of K, 37% of Cu and 65% of B foliar contents were observed in the plot with N fertilization. In site 2, N fertilization decreases foliar contents of 12% P and 32% Ca and increase 10% of SSY and 14% of SY. In site 5, an increase of 11% of SSY, 9% of SY, 39% of Mg and 28% of Fe with N fertilization were observed. In the site’s average of the first year, there was an increase in SSY and SY of 7% and 8%, respectively, and the micronutrients Cu and B had a 16 and 12% lower leaf content in the plots that received nitrogen fertilization (Table 3).

Table 3. Relation with the N fertilized plot and control in each site and in the Year 1 and 2 average. The average was compared by test T contrast.

Site	1	2	3	4	5	Year 1	1	2	3	4	5	Year 2
	--- p valor --											
SSY	6%	10%	-1%	10%	11%	**7%***	-2%	-8%	3%	0%	**-10%***	-4%
TRS	-1%	3%	1%	-1%	-1%*	0%	-2%	-2%	-3%	-1%	-3%	**-2%***
SY	5%	14%	1%	9%	9%*	8%*	-5%	-10%	-1%	-1%	**-14%***	**-7%***
Height	8%	-6%	2%	1%	-1%	1%	-1%	-9%*	6%	0%	1%	-1%
DIAM	2%	3%	-1%	-1%	-1%	0%	2%	-3%	1%	-3%	-5%	-2%
SPAD	**27%***	1%			-7%	7%		-5%		-2%	0%	-2%
N	-4%	3%	0%	13%	-9%	1%		-3%	5%		-3%	0%
P	-10%	**-12%***	3%	-6%	32%	0%		-8%	2%		-6%	-1%
K	**-14%***	-1%	1%	1%	5%	-2%		-5%	-1%		-1%	-2%
Ca	-10%	**-32%***	6%	-3%	34%	-1%		-13%	-3%		3%	-4%
Mg	-14%	-14%	0%	11%	**39%***	4%		-13%	-1%		0%	-5%
S	-7%	-2%	-11%	1%	4%	-2%		**-8%***	**2%***		2%	-1%
Fe	-10%	0%	1%	3%	**28%***	5%		-5%	4%		0%	2%
Mn	-26%	-5%	-14%	12%	11%	-2%		-6%	-11%		3%	-5%
Zn	-14%	1%	-2%	-6%	-5%	-4%		**-7%***	2%		-8%	-5%
Cu	**-37%***	20%	-26%	-26%	-17%	**-16%***		-5%	8%		-5%	-1%
B	**-65%***	-9%	7%	-11%	-7%	**-12%***		-2%	-47%		-17%	-13%
Mo	-1%	21%	-29%	-23%	-42%	-16%		-30%	-4%		**-28%***	**-21%**

1 SSY, sugarcane stalk yield; 2 TRS, theoretical recoverable sugar; 3 sugar yield. *, means different at t-student test using p<.1. Dark red, < -10%; light red, -10% > x > -5%; white, -5% < x < -5%; light green, 5 > x > 10; dark green, >10%. Due to operational limitations, site 1 and 4 were not sampled in the second year.

In year 2, site 2 had a 9% stalk height loss with N fertilization. Site 5 had 10% of SSY loss with the N fertilization, the opposite of the first year. In the second year, there was no SSY response to nitrogen fertilization in ratoon (Figure 7), although there was a loss of 7% SY (Table 3).

The first-year yield gain was 7 Mg ha^{-1} SSY and 1.1 Mg ha^{-1} SY. In the second year, nitrogen fertilization disfavored the sugarcane ripeness. On average, the unfertilized sugarcane presented more than 4 kg sugar Mg^{-1} stalk. Due to the lack of yield response of stalks and the increase in sugar concentration in year 2, N fertilization generated a negative response in sugar yield of 1 Mg ha^{-1} SY (Figure 8).

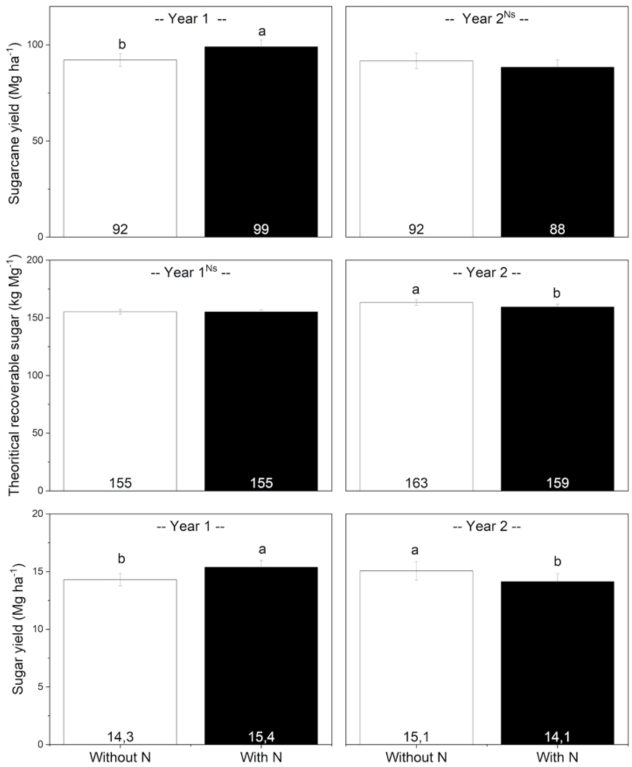


Figure 8. Average of sugarcane stalk yield, theoretical recoverable sugar, and sugar yield for plots receiving or not soil N fertilizer. Average was compared by the t-test using $p < 0.1$.

3.3.4 N and Mo interaction in the yield parameters

The combined analysis of the data indicated that N rates higher than 5 kg ha^{-1}, impaired the stalk yield in the first year of the experiment, which had intense drought after foliar application. However, the rate of 5 kg ha^{-1} N despite being significantly equal to the control, showed a higher yield (2 Mg ha^{-1} SSY higher compared to the control) and the rates of 10 and 20 kg ha^{-1} N showed 5 Mg ha^{-1} SSY lower compared to the control, demonstrating possible toxicity due to high rates of foliar N (Figure 9). There was no interaction between Mo and N at any location and on average. Sites 3 and 4 showed N rates response without Mo effect, where rates 0 and 5 kg ha^{-1} N generated the highest stalk yield (Figure 9).

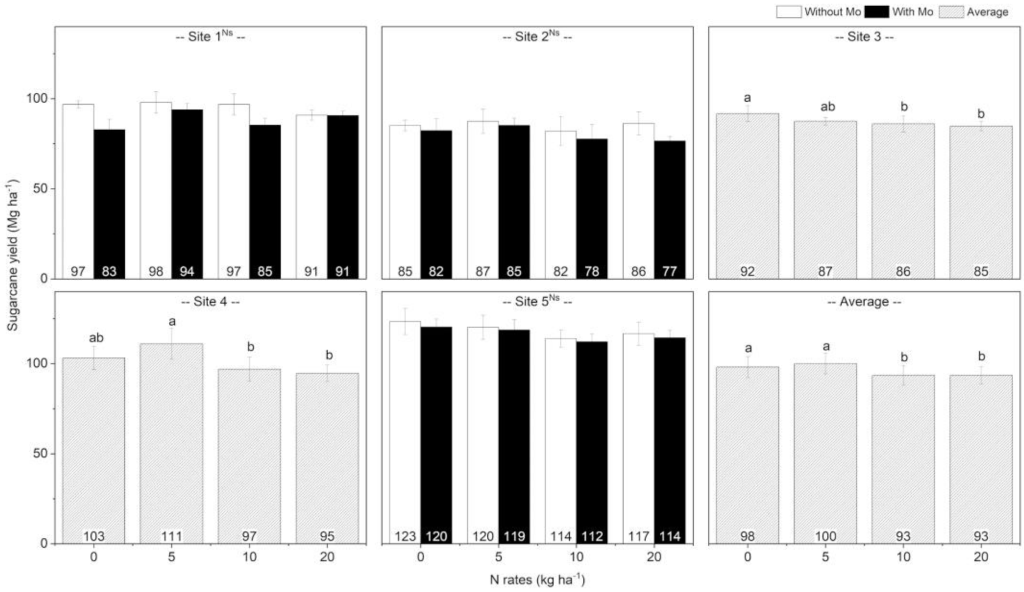


Figure 9. Sugarcane stalk yield in the first crop season. Sites 1, 2 and 5 were not significant. Sites 3, 4 and average were presented as average because there was no interaction between rates. Means were compared by LSD test using $p < 0.1$.

In the first year, immediately after spraying the fertilizers, there was a drought stress of about 30 days in all the center-south region of Brazil, and December and January totaling 182 mm on average of 5 sites. On the other hand, in the second year of the experiment rained an average of 342 mm in the two months that included foliar fertilization, i.e., an increase of 53% more precipitation. In the second year, the N rate of 5 kg ha^{-1} N presented the highest yield, while the rates of 10 and 20 kg ha^{-1} N were equal to the control. Even in the most favorable year for foliar application, rates of 10 and 20 kg ha^{-1} of N did not show a yield response (Figure 10).

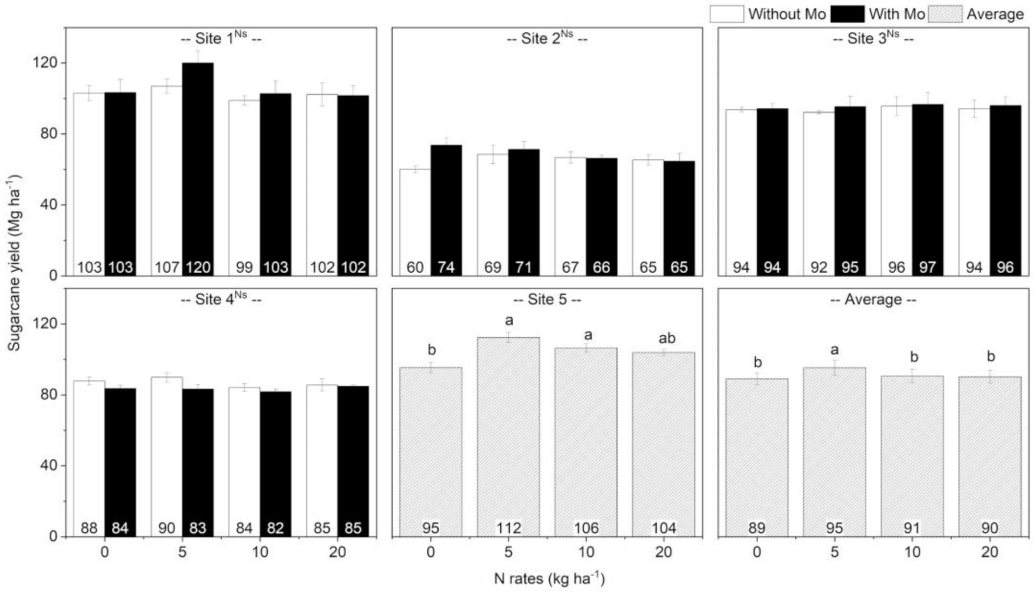


Figure 10. Sugarcane productivity in the second crop season. Sites 1.2.3 and 4 were not significant. Site 5 and average were presented as average because there is no interaction between rates. Means were compared by LSD test using $p < 0.1$.

In the first year, the application of Mo reduced the yield by an average 2 Mg ha^{-1} and site 1 showed a reduction of 8 Mg ha^{-1} with the Mo foliar application. In the average of second year, there was no yield difference with Mo application. In addition, site 4 in the second year, showed a negative response of 4 Mg ha^{-1} SSY with Mo foliar fertilization (Figure 11).

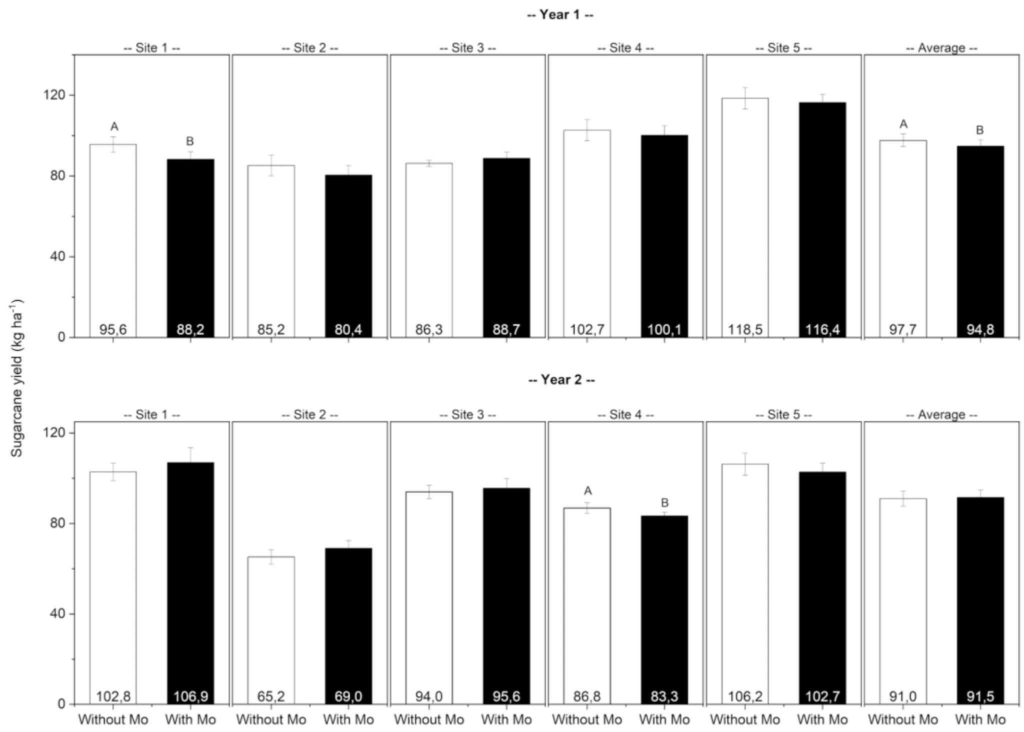


Figure 11. Sugarcane yield in average of with and without Mo fertilized plots in the Years 1 and 2. Error bar represent 90% of confidence level. Means were compared by LSD teste using p< .1.

There was a significant N and Mo interaction in the first year on the TRS average. The 5 kg ha^{-1} N rate without Mo application showed the highest TRS value. Although there is no significant difference, the TRS was higher (1.2 kg sugar Mg^{-1} stalks) in the average plot with Mo application. Site 3 in the first year, site 5 in the second year and the average of the second year showed an increase of TRS with foliar application (0.7 kg sugar Mg^{-1} stalk) (Figure 12).

On average, total sugar yield followed the same behavior as stalk yield. In the first year the yield was higher at rates 0 and 5 kg ha^{-1} N and in the second year, 5 kg ha^{-1} N, was the better rate for the sugar yield.

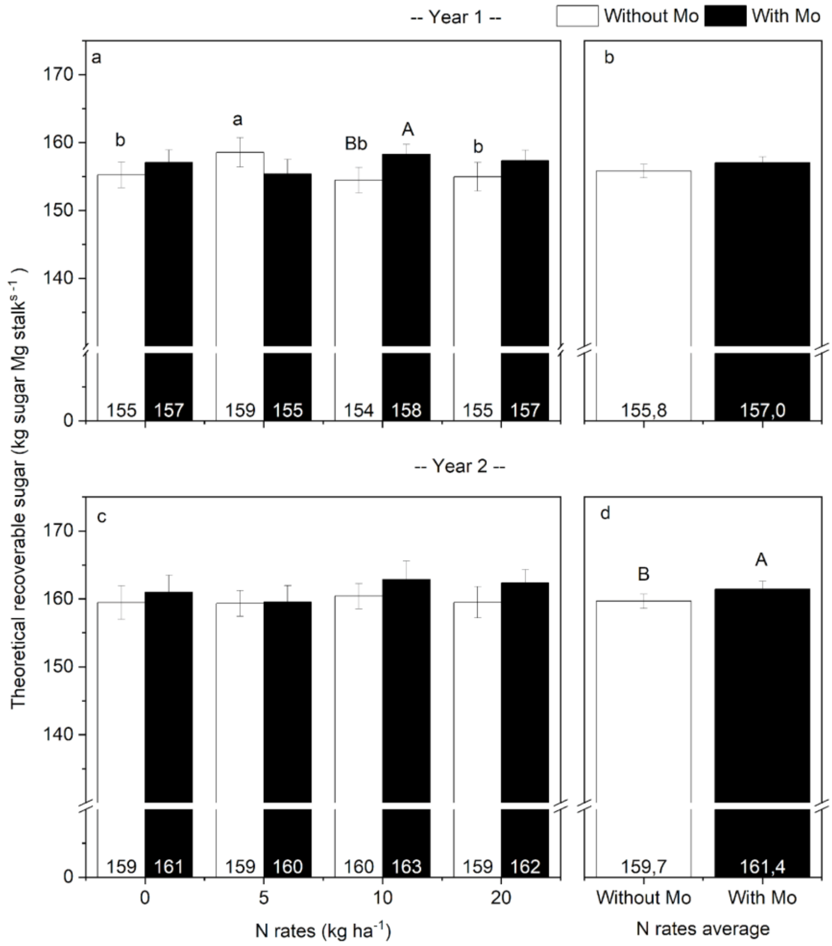


Figure 12. Theorical recoverable sugar influenced by average of N rates and in the average of influence of Mo in the Year 1 and 2. Error bars represent 90% of confidence level. Means were compared by LSD teste using p< .1.

3.4 Discussion

3.4.1 Exploratory analysis

The nutritional sugarcane status (i.e., macronutrients and micronutrients contents) was able to separate areas into clusters in both cycles, first and second year. In the first year, the high SY, SSY, TRS, N, Ca, Fe, Mg, B, heigh, and diameter were variables classified as strong in PCA1. This result is directly associated with site 5, which had the highest yield (116 Mg ha^{-1} SSY). Site 2, on the other hand, had a strong correlation with high levels of Mn, Zn, and Cu, and a negative correlation with SSY. For the second year, the strong correction of SSY, SY, Ca, Mg, and Fe was again observed as strongly correlated variables. In addition, there were also positive highlights of first dimension and negative correlations with high levels of Mn, Zn, and Cu contents and low yield due to site 2.

The negative correlations between SSY, Ca, Mg, N, and S contents with the metallic micronutrients Cu, Mn, and Zn, can be directly linked to soil acidity. Site 5 had Ca contents above V>70%, 25 mg dm^{-3} Ca, and pH>5.0 up to 1 m depth, while site 2 had the same characteristics only in the first layer suitable only in the first layer. There was a Ca negative correlation in the first year with the metal micronutrients Cu, Zn and Mn (mean of - 0.21). In the second year, the negative correlation between Ca versus Cu, Zn, Mn intensified to an average of -0.68. Therefore, the first dimension means high yield has a negative correlation with soil acidity.

3.4.2 N and Mo influence on nutritional status

Under the study conditions, no increases in N content were observed with foliar application in the first year. There was a decrease in N content with the application of a rate of 20 kg ha^{-1} N without Mo in the first year. In the second year, just site 5 showed an increase in leaf N regardless of the Mo application (Table S7).

Environmental factors, such as temperature and relative humidity are decisive in the performance of foliar absorption. Under high relative humidity, cuticle hydration increases, drying of salts deposited on the leaf surface is reduced providing greater foliar absorption. The increase in temperature accelerates the speed of evaporation of the solution, decreases the viscosity, surface tension, thus decreasing foliar absorption. The source selected for the application is another crucial aspect of foliar fertilization (Fernández, Sotiropoulos and Brown, 2015).

Several N sources can be used in foliar application, the most common are urea, ammonium nitrate, and ammonium sulfate. Urea is the most used source of N due to its fast and efficient assimilation (Bi and Scagel, 2008; Bondada et al., 2001; Dong et al., 2002; Yildirim et al., 2007). Despite the N content not having been increased under the study conditions, several works in literature report the fast foliar absorption of N-urea. Trivelin et al. (1988), studied leaf N uptake in sugarcane under controlled conditions found a ^{15}N-urea fast absorption by the sugarcane. The authors performed a foliar application with 5 kg ha^{-1} of N 30 days after planting and the absorption was 50% in the 6h. Because the content was not affected and the isotopic analyses revealed quick foliar absorption, the authors concluded that total content was not an appropriate indicator of absorption.

It's likely that N was transported to young tissues of the leaves due to the high mobility of N in plants and the higher absorption of N-urea, as there was no increase in N detected at the sampling time. Ruan and Gerendás (2015), studying the speed of absorption and translocation of N-urea applied to *Camellia sinenes* L., the results showed the translocation of absorbed N occurs at a significant rate from a day after application. As in our study, the leaves was sampled 30 days after treatment, the N content rise non-detection may be associated with N translocation.

Another point that can explain the lack of N content increase detections is that plants can emit ammonia (NH_3) into the environment. The NH_3 gaseous exchanges by the leaves occur by diffusion, and the direction (i.e., absorption or emission) is determined by the NH_3 concentration in the atmosphere and the ammonium compensation point (ACP) (Farquar et al., 1989; Husted and Mattsson, 1996). ACP is defined by the foliar ammonia concentration in which absorption and emission are equivalent and the ACP depend on numerous factors such as water condition, photoperiod, species, and phenological stage, among others (Holtan-Hartwig and Bockman, 1994; Husted, Mattsson and Schjoerring, 1996; Mattsson et al., 1997). As the sampling time after the application was 30-40 days, it is possible that in addition to the N translocation, the ACP compensated the foliar N concentration and rebalanced the N levels.

The current study has shown that some nutrients were reduced as a result of the application of N rates. The decrease in levels of various nutrients, including P, K, Ca, Mg, Fe, Zn, and Cu, was affected by rates of 10 and 20 kg ha^{-1} N in at least one site or in the average of one of the years. Given that fertilization reduced the amounts of numerous other nutrients, N may have been diluted as a result of the increased stimulation for leaf formation.

In contrast to the N, the application of Mo raised the Mo contents in all sites. The increments in grades ranged from 47 to 192% which means 0.2 to 1.8 mg kg^{-1}, respectively (Table S18). Several works have also reported a large increase in Mo content with foliar application, up to 640% in sunflower (Skarpa et al., 2013), and up to 90% Mo content in seed bean with foliar application (Vieira. 2014).

The Mo application helped to increase some nutrients such as Ca in site 4 (Year 1) and site 2 (Year 2). As well Mo helped B increase by 270% at site 3 (Year 2) and 200% at site 5 (Year 2) with a significant difference. Considering calcium and boron are linked in the synthesis and growth of new plant tissues, the greater Mo content in treated plants may have promoted leaf formation and expansion.

3.4.3 N soil fertilization response

According to the results, the only yield response to nitrogen fertilizer in the soil observed was on the average of the first year (7 Mg ha^{-1} of SSY or 7.6%) (Table 4). Studies have shown that sugarcane has a low NUE, recovering only 26% of the N provided through fertilizers on average, while 32% is immobilized by microbes and the remaining amounts are lost by ammonia volatilization, denitrification into nitrous oxide, and leaching (Otto et al., 2016). One of the main causes of the poor response to nitrogen fertilizer in sugarcane ratoon in the current study is due to low NUE.

The harvesting system may have been another cause of the lack of response in the second year. The Green Cane Trash Blanket (GCTB) system moderates the response to N fertilization, whereas the straw system can improve soil carbon stock and increase the N release for sugarcane in the medium and long-term (Thorburn et al., 2012; Fortes et al., 2013; Ferreira et al., 2016).

The higher nutrient extraction by the plots with the highest yield rate in the first year may be another reason to non-responsiveness fertilization in the second year. Except for site 3, the magnesium contents were below the threshold of 8 mg dm^{-3} (Raij et al., 1997). The supply of micronutrients was also not taken consideration in base fertilization, which might have reduced the response (Table 2).

Nitrogen fertilization decreases Cu contents by 16% in the average of the first year. Except for site 2, Cu contents were from -14% to -26% with the N fertilization (Table 3). The concentration of Cu in plant tissues varies according to the stage of development of the plants and environmental factors, such as plants under high N surplus need higher Cu content and bioavailability increases with soil acidity (Marschner, 1995).

Another highlight was the B decrease of 12 and 13% in the average of the first and second years, respectively (Table 3). Usually, the greater availability of N generates a negative interaction with B. Increasing N availability significantly decreases the contents and toxic effects of B (Koohakan and Maftou, 2015; Gupta et al., 1981). The interaction of N with B is of great significance because both elements are quite mobile in the soil environment.

Although there is no significant difference on average, the Mo content was 16 and 13% lower with nitrogen fertilization in the first and second year, respectively (Table 3). Thus, indicating lower Mo uptake in the condition of high N supply.

3.4.4 N and Mo spray on the yield parameters

After the spray, the first year of the experiment featured a significant water deficit. On the sites average, the first-year received 47% fewer rainfalls than the second-year over the 60 days subsequent the spray. In the first year, sites 3, 4 and sites average, rates of 0 and 5 kg ha^{-1} of N generated the highest yields. Although there is no statistical significance, the rate of 5 kg ha^{-1} N was the rate with the highest yield in sites 1 and 2. Yield reduction effects with rates 10 and 20 kg ha^{-1} N were observed in sites 3 and 4 (Figure 3). The highest yield response was produced by the rate of 5 kg ha^{-1} N in the second year (6.2 Mg Ha^{-1} SSY) (Table 4).

There are several reasons why high rates of foliar applied N may have shown a negative or non-response. Among them are: (a) The breakdown of the leaf cuticle's surface tension caused by the application of urea may result in water loss from the leaf and reduced pathogen defense; (b) Increased evapotranspiration results from increased leaf area development by the stimulation of nitrogen fertilization; (c) the possible toxicity caused by the increase in ammoniacal ions; (d) the alkalinization of cell pH and conductive vessels due to urea hydrolysis, which can accelerate the dissociation of abscisic acid (ABA), shifting ABAH to ABA^- and facilitating its transport between membranes to guard cells (Taiz et al., 2017)

Oliveira (2011), found the maximum N accumulation rate value i.e., daily rate at peak absorption of 655 g day^{-1} N in ratoon cane with the productivity of 93 Mg ha^{-1} SSY, i. e., very close yield to that obtained in this work, if considering 75% foliar N-fertilizer absorption, the N supply would represent 5.8, 11.5 and 23.1 absorption days, respectively, for the rates of 5. 10 and 20 kg ha^{-1} N. It can be a challenge for the plant to receive and store this amount of N, especially the rates of 10 and 20 kg ha^{-1}. Thus, these results demonstrate that rates above 5 kg ha^{-1} N can be dangerous for the crop.

Molybdenum caused a yield reduction of 3 Mg ha^{-1} SSY in the first year and did not change yield in the second year. The results of yield loss and a TRS increase caused by the application of Mo, can be explained by its role as a cofactor of abscisic-aldehyde oxidase enzymes (AAOs). The increase in water restriction generates signaling to produce ABA in the tissue and consequently increases stomatal resistance (Wilkinson and Davies, 1997). The increase of ABA in the plant promotes leaf senescence, therefore promoting sugarcane ripeness, which explains the higher TRS.

One of the current work’s hypotheses was that the Mo application could further increase sugarcane's NUE because of the crucial functions played by nitrate reductase in the assimilation of nitrogen by plants. The nitrate reductase is sensitive to environmental elements such light, carbon dioxide, concentrations of nitrate and nitrogen compounds, phytohormones, and carbon compounds and has complicated regulation (Taiz et al., 2017).

In addition to the complexity of nitrate reductase regulation expression and activity are seasonal in each tissue, culture stage, and environmental condition. Nitrate reductase activity peaks also during the maximum period of leaf expansion and becomes very low with fully expanded leaves (Marschner, 1995). Interaction studies between N, Mo and varieties, with the application of N and Mo in the planting furrow have shown that regardless of N, Mo or genotype was used, the nitrate reductase activity peaked in the leaves was in 100 days after planting and then decrease for the remaining until the null activity at 200 days of the cycle Santos et al. (2019). Therefore, in our studies, the enzyme activity could already be low or null when the application was carried out since the application period was at least 150 days after the previous harvest.

3.5 Conclusion

Soil application of 0.8 kg N t^{-1} of stalks presented a limited effect on sugarcane yield in both crop seasons. In opposite, foliar spray with N presented a potential to increases sugarcane yield by 6 t ha^{-1} on the average of the five sites evaluated herein. The effects of N foliar spray on sugarcane yield were much more consistent in the season 2019/2020 rather than in the 2018/2019 crop season, and this can be attributed to better rainfall distribution in the 2019/2020 crop season. This is an indicator that response of sugarcane to foliar N spray are dependent upon weather conditions following application. Using rates of N higher than 5 kg ha-1 N in foliar spray does not show potential in further improving sugarcane yield.

Molybdenum showed a limited potential in increasing sugarcane yield in association with foliar N application. In opposite, it caused reduction in yield in some sites. The Mo application caused an improvement in the maturation of sugarcane and this effect might be further addressed in future studies

Results of this study demonstrates a potential of using a reduced N rate for soil application (0.8 kg N t-1 of stalks) in comparison to usual recommendations, in addition to a 5 kg ha-1 N foliar spray in the maximum growth stage of the crop to maximize sugarcane yield.

References

AMARAL, L. R.; MOLIN, J. P.; SCHEPERS, J. S. Algorithm for variable-rate nitrogen application in sugarcane based on active crop canopy sensor. **Agronomy Journal**, v. 107, p. 1513-1523, 2015.

BI, G.; SCAGEL, C. F. Nitrogen uptake and mobilization by Hydrangea leaves from foliar-sprayed urea in fall depend on plant nitrogen status. **Horticultural Science**, v. 43, p. 2151–2154, 2008.

BONDADA, B. R.; SYVERTSEN J. P.; ALBRIGO, L. G. Urea nitrogen uptake by citrus leaves. **Horticultural Science**, v. 36, p. 1061–1065, 2001.

BOSCHIERO, B. N. **Adubação nitrogenada em soqueiras de cana-de-açúcar: influência**

do uso em longo prazo de fontes e/ou doses de nitrogênio. Tese. Escola Superior de Agricultura "Luiz de Queiroz", ESALQ/USP, Brasil. 2017.

CAMARGO, A. O.; MONIZ, A. C.; JORGE, J. A.; VALADARES, J. M. A. S. (2009). **Métodos de análise química, mineralógica e física de solos do Instituto Agronômico de Campinas**. Campinas, Brazil: Instituto Agronômico de Campinas.

CAMPBELL, M. K.; FARRELL, S. O. **Bioquímica**. São Paulo: Cengage Learning, 2016.

CANTARELLA, H.; TRIVELIN, P. C. O.; CONTIN, T. L. M.; DIAS. F. L. F.; ROSSETTO, R.; MARCELINO, R.; COIMBRA, R. B.; QUAGGIO, J. A. Ammonia volatilization from urease inhibitor-treated urea applied to sugarcane trash blankets. **Scientia Agricola**, v. 65. p.397-401. 2008.

CANTARELLA, H.; TRIVELIN. P. C. O.; VITTI. A. C.; Nitrogênio e enxofre na cultura da cana-de-açúcar. In: YAMADA. T.; ABDALLA. S. R. S.; VITTI, G. C. (Ed.) **Nitrogênio e enxofre na agricultura brasileira.** 1.ed. Piracicaba: IPNI Brasil. 2007. p. 407-464.

CASTRO, S. G. Q.; DECARO, S. T.; FRANCO, H. C. J.; MAGALHÃES, P.G.; GARSIDE, A. L.; MUTTON, M. A. Best practices of nitrogen fertilization management for sugarcane under green cane trash blanket in Brazil. **Sugar Tech**, v. 19, p. 51-56, 2016.

CASTRO, S. G. Q.; MAGALHÃES, P. S. G.; CASTRO, S. A. Q.; KÖLLN, O. T.; FRANCO, H. C. J. Optimizing Nitrogen Fertilizer Rates at Distinct In-season Application Moments in Sugarcane. **International Journal of Plant Production**, v. 1, p. 1-16, 2021.

CASTRO, S. A. Q. **Aproveitamento do N-fertilizante (N-ureia) pela cana-de-açucar aplicado por via foliar no período de máximo crescimento da cultura em complemento à adubação de solo**. 2022, 22p. Tese (doutorado) – Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 2022.

CHENG, L.; DONG, S.; BATES, T. Urea uptake and nitrogen mobilization by apple leaves in relation to tree nitrogen status in autumn. **Journal of Horticultural Science & Biotechnology**, v.77, n. 1, 77, p. 13–18, 2002.

COLETI, J. T.; CASAGRANDE. J. C.; STUPIELLO, J. J.; RIBEIRO, L. D.; OLIVEIRA, G. R. **Remoção de macronutrientes pela cana-planta e cana-soca. em Argissolos. variedades RB835486 e SP813250**. STAB. Açúcar. Álcool e Subprodutos. Piracicaba, v. 24. n. 5. p. 32-36. 2006.

CONTIN, T. L. M. **Ureia tratada com inibidor da urease NBPT na adubação da cana-de-açúcar colhida sem despalha a fogo**. Campinas, Instituto Agronômico de Campinas, 2007. 72p. (Dissertação de Mestrado).

COSTA, M. C. G.; VITTI, G. C.; CANTARELLA, H. Volatilização de N-NH_3 de fontes nitrogenadas em cana-de-açúcar colhida sem despalha a fogo. **Revista Brasileira de Ciência do Solo**, v. 27, n. 4, p. 631–637, 2003.

CRUTZEN, P. J.; MOSIER, A. R.; SMITH, K. A.; WINIWARTER, W. N_2O Release from Agrobiofuel Production Negates Global Warming Reduction by Replacing Fossil Fuels. **Atmospheric Chemistry Physics**, v. 8, p. 389-395, 2008.

DONG, S. F.; CHENG. L. L.; SCAGEL. C. F.; FUCHIGAMI. L. H. Nitrogen absorption. translocation and distribution from urea applied in autumn to leaves of young potted apple (Malus domestica) trees. **Tree Physiology**, v. 22, p. 1305-1310, 2002.

FAGERIA, N. K.; BARBOSA FILHO, M. P.; MOREIRA, A.; GUIMARÂES, C. M. Foliar fertilization of crop plants. **Journal of Plant Nutrition**, v. 23, p. 1044-1064, 2009.

FARQUHAR. G.D.; FIRTH. P.M.; WETSELAAR. R.; WEIR. B. On the gaseous exchange of ammonia between leaves and the environment: Determination of the ammonia compensation point. **Plant Physiology**, v. 66. p. 710-714. 1980.

FERNANDES, A. M.; DE QUEIROZ, A. C.; PEREIRA, J. C.; LANA, R. D. P.; BARBOSA, M. H. P.; DA FONSECA; D. M.; VITTORI, A. Composição químico-bromatológica de variedades de cana-de-açúcar (*Saccharum spp* L.) com diferentes ciclos de produção (precoce e intermediário) em três Idades de corte. **Revista Brasileira de Zootecnia**, v. 32, n. 4, p. 977–985, 2003.

FERNÁNDEZ, V.; EICHERT, T. Uptake of hydrophilic solutes through plant leaves: current state of knowledge and perspectives of foliar fertilization. **Critical Reviews in Plant Science**, v. 28, p. 36–68, 2009.

FERNÁNDEZ, V.; SOTIROPOULOS, T.; BROWN, P. (2015) **Adubação foliar: Fundamentos Científicos e Técnicas de Campo**. Abisolo, São Paulo, 150 p.

FERREIRA, D. A.; FRANCO, H. C. J.; OTTO, R.; VITTI, A. C.; FORTES, C.; FARONI, C. E.; GARSIDE, A. L.; TRIVELIN, P. C. O. Contribution of N from green harvest residues for sugarcane nutrition. **GCB Bioenergy,** v. 8, n. 5, p. 859-866, 2016.

FORTES, C.; VITTI, A. C.; OTTO, R.; FERREIRA, D. A.; FRANCO, H. C. J.; TRIVELIN, P. C. O. Contribution of nitrogen from sugarcane harvest residues and urea for crop nutrition. **Scientia Agricola,** v. 70, p. 313–320, 2013.

FRANCO, H. C. J. **Eficiência agronômica da adubação nitrogenada de cana-planta.** 127p. Tese (Doutorado em Solos e Nutrição de Plantas) – Escola Superior de Agricultura Luiz de Queiroz. Universidade de São Paulo. Piracicaba. 2008.

GUELFI, D. Fertilizantes nitrogenados estabilizados, de liberação lenta ou controlada. **Informações Agronômicas**, v. 157, p. 1-14, 2017.

GUPTA, U. C., AND J. LIPSETT Molybdenum in soil, plants, and animals. **Advances in Agronomy**, v. 34, p. 73–115, 1981.

HOLTAN-HARTWIG, L.; BOCKMAN, O.C. Ammonia exchange between crops and air. **Norwegian Journal of Agricultural Sciences**, v. 14, p. 1-41, 1994.

HUSTED. S.; MATTSSON. M.; SCHJOERRING. J. K. Ammonia compensation points in two cultivars of *Hordeum vulgare* L. during vegetative and generative growth. **Plant, Cell and Environment**, v. 19. p. 1299-1306. 1996.

JAISWAL, D.; DE SOUZA, A. P.; LARSEN, S.; LEBAUER, D. S.; MIGUEZ, F. E.; SPAROVEK, G.; BOLLERO, G.; BUCKERIDGE, M. S.; LONG, S. Brazilian sugarcane ethanol as an expandable green alternative to crude oil use. **Nature Climate Change**, v. 7, p. 788-792, 2017.

KLEIN, I.; WEINBAUM, S. A. Foliar application of urea to olive, translocation of urea nitrogen as influenced by sink demand and nitrogen deficiency **Journal of American Society Horticultural Science**, v. 109, p. 356–360, 1984.

LEA-COX, J. D.; SYVERTSEN, J. P. Nitrogen uptake by Citrus leaves. **Journal of American Society Horticultural Science**, v. 120, p. 505–509, 1995.

MALAVOLTA, E.; VITTI, G. C.; OLIVEIRA, S. A. **Avaliação do estado nutricional das plantas**: princípios e aplicações. 2.ed. Piracicaba: Associação Brasileira para Pesquisa da Potassa e do Fosfato, 1997. 319 p.

MALAVOLTA. E. **Elementos de nutrição mineral de plantas**. São Paulo. Editora Agronômica Ceres Ltda. 1980. 251p.

MARIANO, E.; TRIVELIN, P. C. O.; VIEIRA, M. X.; LEITE, J. M.; OTTO, R.; FRANCO, H. C. J. Ammonia losses estimated by an open collector from urea applied to sugarcane straw. **Revista Brasileira de Ciência do Solo**, v. 36, p. 411-419, 2012.

MARKWELL, J.; OSTERMAN, J. C.; MITCHELL, J. L. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. **Photosynthesis Research**, v. 46, p. 467-472, 1995.

MARSCHNER, H. Mineral Nutrition of Higher Plants. 2nd Edition, Academic Press, London, 1995, 645 p.

MATTSSON, M.; HAUSLER, R. E.; LEEGOOD, R. C.; SCHJOERRING, J. K. Leaf-atmosphere NH_3 exchange in barley mutants with reduced activities of glutamine synthetase. **Plant Physiology**, Waterbury, v. 114, p. 1307-1312, 1997.

MELLIS, E. V.; QUAGGIO, J. A.; BECARI, G. R. G.; TEIXEIRA, L. A. J.; CANTARELLA, H.; DIAS, F. L. F. Effect of micronutrients soil supplementation on sugarcane in different production environments: Cane plant cycle. **Agronomy Journal**, v. 108, p. 2060–2070, 2016.

MIRA, A. B.; CANTARELLA, H.; SOUZA-NETTO, G. J. M.; MOREIRA, L. A.; KAMOGAWA, M. Y.; OTTO, R. Optimizing urease inhibitor usage to reduce ammonia emission following urea application over crop residues. **Agriculture Ecosystems & Environment**, v. 248, p. 105-112, 2017.

OLIVEIRA, E. C. A. O. **Balanço nutricional de cana-de-açúcar relacionado à adubação nitrogenada**. Tese (Doutorado em Solos e Nutrição de Plantas) – Escola Superior de Agricultura Luiz de Queiroz. Universidade de São Paulo. Piracicaba. 2011.

OTTO, R.; CASTRO, S. A. Q.; MARIANO, E; CASTRO, S G. Q.; FRANCO, H. C. J.; TRIVELIN, P. C. O. "Nitrogen use efficiency for sugarcane-biofuel production: what is next?" **BioEnergy Research,** v. 9, p. 1272-1289, 2016.

OTTO, R.; MULVANEY, R. L.; KHAN, S. A.; TRIVELIN, P. C. O. Quantifying soil nitrogen mineralization to improve fertilizer nitrogen management of sugarcane. **Biology and Fertility of Soils**, v. 49, n. 7, p. 893–904, 2013.

OTTO, R.; SOUZA-NETTO, G. J. M.; FERRAZ-ALMEIRA, R.; ALTARUGIO, L.; FAVARIN, J. L. Multisite response of sugarcane to nitrogen rates and split application under Brazilian field conditions. **Agronomy Journal**, v. 113, p 419-435, 2020.

RAIJ, B. V.; ANDRADE, J. C.; CANTARELLA, H.; QUAGGIO, J. A. (2001). **Análise química para avaliação de fertilidade de solos tropicas**. Campinas: Instituto Agronômico de Campinas.

RAIJ, B. V.; CANTARELLA, H.; QUAGGIO, J. A.; FURLANI, A. M. C. **Recomendações de adubação e calagem para o Estado de São Paulo**. 2.ed. Campinas: Instituto Agronômico: Fundação IAC, 1997 (IAC. Boletim técnico, 100), 285 p.
RUAN, J; GERENDÁS, J. Absorption of foliar-applied urea-15N and the impact of low nitrogen, potassium, magnesium and sulfur nutritional status in tea (*Camellia sinensis* L.) plants. **Soil Science and Plant Nutrition,**,v. 61, p. 653–663, 2015.

SANTOS, R. L. **Molibdênio no metabolismo e fixação biológica de N na cana-de-açúcar**. Tese. Universidade Federal Rural de Pernambuco, Brasil, 2014.

SANTOS, R.L.; FREIRE, F.J.; OLIVEIRA, E.C.A.; TRIVELIN, P. C. O.; FREIRE, M. B. G. S.; BEZERRA, P. C.; OLIVEIRA, R. I.; SANTOS, M. B. C.Changes in Biological Nitrogen Fixation and Natural-Abundance N Isotopes of Sugarcane Under Molybdenum Fertilization. **Sugar Tech**, v. 21, p. 925–93, 2019.

SILVA, A. G. B.; SEQUEIRA, C. H.; SERMARINI, R. A.; OTTO, R. Urease Inhibitor NBPT on Ammonia Volatilization and Crop Productivity: A Meta-Analysis. **Agronomy Journal**, v. 109, p. 1-3, 2016.

SILVA, M. J.; FRANCO, H. C. J.; MAGALHÃES, P. S. G. Liquid fertilizer application to ratoon cane using a soil punching method. **Soil & Tillage Research**, v. 165, p. 279-285, 2017.

ŠKARPA, P; KUNZOVÁ, E.; ZUKALOVÁ H. Foliar fertilization with molybdenum in sunflower (*Helianthus annuus* L.). **Plant Soil Environmental,** v. 59, n. 4, p 156-161, 2013.

Soil Survey Staff. (2022). **Keys to soil taxonomy** (13th ed.). Washington, DC: USDA-NRCS.

TAIZ, L.; ZEIGER, E.; MOLLER, I.; MURPHY, A. **Fisiologia e desenvolvimento vegetal**. 6.ed. Porto Alegre: Artmed, 2017. 888 p.

TASSO JUNIOR, L. C.; MARQUES, M. O.; CAMILOTTI, F.; SILVA, T. Extração de macronutrientes em cinco variedades de cana-de-açúcar cultivadas na região centro norte do Estado de São Paulo. STAB. **Açúcar Álcool e Subprodutos**. Piracicaba. v. 25., n. 6, p. 38-42, 2007.

THAPA, U.; PRASAD, P. H.; RAI, R. Studies on Growth, Yield and Quality of Broccoli (*Brassica Oleracea* L. Var Italica Plenck) as Influenced by Boron and Molybdenum. **Journal of Plant Nutrition**, v. 39, n. 2, p. 261–267, 2016.

THORBURN, P; MEIER, E. A.; COLLINS, K.; ROBERTON, F. A. Changes in soil carbon sequestration. fractionation and soil fertility in response to sugarcane residue retention are site-specific. **Soil Tillage Research,** v. 120, p. 99–111, 2012.

TRIVELIN, P. C. O.; CARVALHO, J. G.; SILVA A. Q.; PRIMAVESI, A. C. P. A.; CAMACHO, E.; EIMORE, I. E.; GUILHERME, M. R. **Adubação foliar de cana-de-açúcar (*Saccharum* spp) absorção e translocação de uréia-N.** Energia Nuclear na Agricultura. Piracicaba, v. 9, p. 52-65, 1988.

TRIVELIN, P. C. O.; CARVALHO, J. G.; SILVA, A. Q.; PRIMAVESI, A. C. P. A.; CAMACHO, E.; EIMORI, I. E. GUILHERME, M. R. Adubação foliar de cana-de-açúcar (*Saccharum* spp): Absorção e translocação de ureia-15N. **Energia Nuclear na Agricultura**, v. 9, p. 52-65, 1988.

VIEIRA, R. F.; de PAULA, T. J.; CARNEIRO, J. E. D.; QUEIROZ, M. V. Genotypic Variability In Seed Accumulation Of Foliar-Applied Molybdenum to Common Bean Volume. **Revista Brasileira de Ciência do solo**, v. 38, n. 1, p. 205-213, 2014.

VILLALBA, H. A. G.; LEITE, J. M.; OTTO, R.; TRIVELIN, P. C. O. Fertilizantes nitrogenados: novas tecnologias. **Informações Agronômicas**, v. 148, p. 12-18, 2014.

VITTI, A. C.; TRIVELIN, P. C. O.; GAVA, G. J. C.; FRANCO, H. C. J.; BOLOGNA, I. R.; FARONI, C.E. Produtividade da cana-de-açúcar relacionada à localização de adubos nitrogenados aplicados sobre os resíduos culturais em canavial sem queima. **Revista Brasileira de Ciência do Solo**, v. 31, p. 491-498, 2007.

WEI, L. P.; LI, Y. R.; YANG, L. T. Effects of molybdenum on nitrogen metabolism of sugarcane. **Sugar Tech**, v. 9, n. 1, p. 36–42, 2007.

WILKISON, S.; DAVIES. W. J. Xylem sap pH increase: A drought signal received at the apoplastica face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast. **Plant Physiology**, v. 113, p. 559-573, 1997.

YILDIRIM, E.; GUVENC, I.; TURAN, M.; KARATAS, A. Effect of foliar urea application on quality, growth, mineral uptake and yield of broccoli (*Brassica oleracea* L., var. italica). **Plant Soil and Environment**, v. 53, p. 120-128, 2007.

Supplementary material

Table S1. Sugarcane Stalk Yield of the 5 sites and the annual average for the year 1 and 2.

Soil	**N**	**Mo**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 1**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 2**
kg t^{-1}	**kg ha^{-1}**	**g ha^{-1}**	**--- t ha^{-1} SSY (Year 1) ---**						**--- t ha^{-1} SSY (Year 2) ---**					
-	-	-	91.2	77.7	88.1	92.3	111.4	92.1	105.5	65.5	91.2	87.6	108.4	91.6
0.8	0	-	96.8	85.2	87.6	102.0	123.3	99.0	103.0	60.1	93.7	87.8	97.4	88.4
0.8	5	-	98.0	87.5	86.8	114.9	120.1	101.5	106.9	68.5	92.2	89.8	117.8	95.1
0.8	10	-	96.9	82.0	84.4	98.4	113.9	95.1	98.9	66.8	95.7	84.1	108.9	90.9
0.8	20	-	90.9	86.3	86.3	95.4	116.6	95.1	102.2	65.3	94.2	85.5	100.8	89.6
0.8	0	100	82.8	82.3	95.9	104.3	120.4	97.1	103.3	73.7	94.3	83.6	93.2	89.6
0.8	5	100	93.9	85.2	88.1	107.1	118.7	98.6	120.0	71.3	95.4	83.3	107.0	95.4
0.8	10	100	85.4	77.7	87.6	95.4	112.1	91.6	102.8	66.4	96.6	81.7	103.9	90.3
0.8	20	100	90.7	76.6	83.3	93.8	114.3	91.7	101.7	64.7	96.0	84.8	106.9	90.8
N - Mo			95.6 A	85.2	86.3	102.7	116.4	97.7 A	102.8	65.2	94.0	86.8 A	106.2	91.0
N + Mo			88.2 B	80.4	88.7	100.1	118.5	94.8 B	106.9	69.0	95.6	83.3 B	102.7	91.5
0			89.8	83.7	91.7 a	103.1 ab	121.9	98.0 a	103.2	66.9	94.0	85.7	95.3 b	89.0 b
5			95.9	86.3	87.5 ab	111.0 a	119.4	100.0 a	113.5	69.9	93.8	86.6	112.4 a	95.2 a
10			91.1	79.8	86.0 b	96.9 b	113.0	93.4 b	100.8	66.6	96.1	82.9	106.4 a	90.6 b
20			90.8	81.5	84.8 b	94.6 b	115.4	93.4 b	101.9	65.0	95.1	85.2	103.8 ab	90.2 b
p_Nsoil_contrast			0.4	0.27	0.93	0.15	0.18	0.04*	0.67	0.37	0.97	0.97	0.06*	0.22
p_Mo			0.02*	0.26	0.17	0.49	0.57	0.05*	0.31	0.16	0.64	0.05	0.33	0.67
p_Nrate			0.47	0.70	0.06*	0.02*	0.36	<0.01*	0.38	0.62	0.16	0.47	0.04*	0.02*
p_Nrate_Mo			0.34	0.91	0.18	0.99	0.99	0.95	0.14	0.21	0.21	0.64	0.44	0.97
p_site								<0.01*						<0.01*
p_Site_Mo								0.15						0.25
p_Site_Nrate								0.60						0.24
p_Site_Mo_Nrate								0.91						0.61

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters rmeans N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S2. Theoretical recoverable sugar of the 5 sites and the annual average for the year 1 and 2.

Soil	**N**	**Mo**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 1**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 2**
kg t^{-1}	**kg ha^{-1}**	**g ha^{-1}**	**--------------------- kg t^{-1} TRS (Year 1) ---------------------**						**--------------------- kg t^{-1} TRS (Year 2) ---------------------**					
-	-	-	159.8	156.1	140.6	161.9	157.9	155.3	162.6	159.8	164.8	149.0	180.4	163.3
0.8	0	-	158.1	160.3	142.3	159.6	156.0	155.3 b	158.8	157.1	159.1	147.0	175.4	159.5
0.8	5	-	161.1	156.3	143.5	165.1	166.8	158.6 a	161.9	157.0	159.0	147.8	171.0	159.3
0.8	10	-	155.8	156.6	144.8	156.9	160.5	154.5 Bb	162.0	153.9	159.3	155.9	171.0	160.4
0.8	20	-	160.7	154.3	139.9	158.1	161.9	155.0 b	157.3	157.5	158.5	148.2	176.0	159.5
0.8	0	100	160.3	157.1	144.7	158.5	164.9	157.1	160.2	155.1	160.7	149.1	179.8	161.0
0.8	5	100	158.7	155.2	141.9	160.6	164.0	154.8	161.8	155.9	159.4	145.2	175.3	159.5
0.8	10	100	158.2	157.9	149.1	162.5	163.8	158.3 A	162.1	156.9	157.6	154.4	183.1	162.8
0.8	20	100	158.7	157.8	147.7	163.2	159.3	157.3	161.1	158.8	160.7	154.8	176.4	162.4
N - Mo			158.9	156.8	142.6 B	159.9	161.3	155.8	160.0	156.4	159.0	149.7	173.3 B	159.7 B
N + Mo			159.0	157.0	145.8 A	161.2	163.0	156.9	161.3	156.7	159.6	150.9	178.6 A	161.4 A
0			159.2	158.7	143.5	159.0	160.4	156.2	159.5	156.1	159.9	148.1 bc	177.6	160.2
5			159.9	155.8	142.7	162.9	165.4	156.7	161.8	156.4	159.2	146.5 c	173.2	159.4
10			157.0	157.2	147.0	159.7	162.1	156.4	162.1	155.4	158.5	155.1 a	177.0	161.6
20			159.7	156.0	143.8	160.7	160.6	156.1	159.2	158.2	159.6	151.5 ab	176.2	160.9
p_Nsoil_contrast			0.66	0.27	0.65	0.53	0.06*	0.30	0.30	0.46	0.13	0.59	0.19	0.02*
p_Mo			0.97	0.91	0.02	0.61	0.47	0.12	0.31	0.87	0.81	0.48	0.01*	0.05*
p_Nrate			0.50	0.51	0.14	0.70	0.41	0.71	0.26	0.98	0.98	<0.01*	0.39	0.31
p_Nrate_Mo			0.53	0.43	0.12	0.39	0.24	0.06*	0.68	0.95	0.95	0.21	0.22	0.71
p_site								<0.01*						<0.01*
p_Site_Mo								0.60						0.36
p_Site_Nrate								0.23						0.28
p_Site_Mo_Nrate								0.39						0.68

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters rmeans N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S3. Sugar Yield of the 5 sites and the annual average for the year 1 and 2.

Soil	N	Mo	Site 1	Site 2	Site 3	Site 4	Site 5	Year 1	Site 1	Site 2	Site 3	Site 4	Site 5	Year 2
kg t^{-1}	**kg ha^{-1}**	**g ha^{-1}**	**--------------------- t ha^{-1} SY (Year 1) ---------------------**						**--------------------- t ha^{-1} SY (Year) ---------------------**					
-	-	-	14.5	12.1	12.4	15.0	17.5	14.3	17.2	10.5	15.0	13.1	19.6	15.1
0.8	0	-	15.3	13.8	12.5	16.2	19.1	15.4	16.3	9.4	14.9	12.9	16.8	14.1
0.8	5	-	15.8	13.7	12.5	18.9	20.0	16.2	17.3	10.7	14.7	13.3	20.2	15.2
0.8	10	-	15.1	12.8	12.2	15.3	18.3	14.7	16.0	10.3	15.3	13.1	18.6	14.7
0.8	20	-	14.6	13.3	12.1	15.1	18.9	14.8	16.1	10.3	14.9	12.7	17.7	14.3
0.8	0	100	13.2	13.0	13.9	16.5	19.8	15.3	16.6	11.4	15.1	12.5	16.8	14.5
0.8	5	100	14.9	13.2	12.5	17.2	19.4	15.3	19.4	11.1	15.2	12.1	18.7	15.3
0.8	10	100	13.5	12.3	13.1	15.5	17.8	14.4	16.7	10.4	15.3	12.6	19.1	14.8
0.8	20	100	14.4	12.1	12.3	15.3	18.2	14.4	16.4	10.3	15.5	13.1	18.8	14.8
N - Mo			15.2 A	13.3	12.3 B	16.4	18.8	15.3 A	16.4	10.2	14.9	13.0	18.4	14.6
N + Mo			14.0 B	12.5	12.9 A	16.1	19.1	14.9 B	17.3	10.8	15.3	12.6	18.4	14.8
0			14.3	13.4	13.2 a	16.4 b	19.5	15.3 a	16.5	10.4	15.0	12.7	16.8 b	14.3 b
5			15.3	13.4	12.5 ab	18.1 a	19.7	15.7 a	18.4	10.9	14.9	12.7	19.4 a	15.3 a
10			14.3	12.5	12.6 ab	15.4 b	18.0	14.5 b	16.3	10.3	15.3	12.9	18.8 a	14.7 b
20			14.5	12.7	12.2 b	15.2 b	18.5	14.7 b	16.2	10.3	15.2	12.9	18.3 ab	14.6 b
p_Nsoil_contrast			0.47	0.12	0.94	0.23	0.03*	0.01*	0.43	0.34	0.93	0.88	0.02*	0.05*
p_Mo			0.02*	0.19	0.05*	0.62	0.59	<0.01*	0.23	0.12	0.61	0.17	0.99	0.27
p_Nrate			0.38	0.78	0.09*	<0.01*	0.16	0.07*	0.11	0.65	0.97	0.92	0.08*	0.05*
p_Nrate_Mo			0.56	0.96	0.18	0.51	0.81	0.78	0.74	0.74	0.98	0.30	0.52	0.93
p_site								<0.01*						<0.01*
p_Site_Mo								0.09*						0.51
p_Site_Nrate								0.26						0.24
p_Site_Mo_Nrate								0.89						0.76

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters rmeans N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S4. Stalk height of the 5 sites and the annual average for the year 1 and 2.

Soil	**N**	**Mo**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 1**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 2**
kg t^{-1}	**kg ha^{-1}**	**g ha^{-1}**	**--------------------- Height - cm (Year 1) ---------------------**						**--------------------- Height - cm (Year 2) ---------------------**					
-	-	-	255.5	281.4	268.2	277.4	354.7	287.4	259.1	258.6	274.6	294.2	269.2	271.1
0.8	0	-	275.1	264.6	274.6	281.2	349.5	289.0	256.9	234.8	289.9	293.7	271.6	269.4
0.8	5	-	262.2	271.8	269.9	295.0	347.8	289.3	256.1	257.5	286.8	290.0	283.6	274.8
0.8	10	-	276.0	287.9	271.9	285.2	351.2	294.4	255.6	248.5	284.4	294.3	264.4	269.4
0.8	20	-	261.4	284.9	263.4	276.7	357.0	288.7	275.4	246.4	289.5	289.8	284.8	277.2
0.8	0	100	249.0	271.4	265.7	280.5	355.6	284.4	281.6	255.5	284.1	286.6	276.9	277.0
0.8	5	100	251.2	279.5	264.6	289.1	351.2	287.1	265.8	261.8	290.5	281.0	282.1	276.2
0.8	10	100	270.1	270.6	265.6	281.6	348.7	287.3	268.6	253.2	284.0	301.1	278.4	277.1
0.8	20	100	253.0	278.4	266.3	289.8	352.8	288.0	277.1	252.6	289.3	292.8	283.9	279.1
N - Mo			268.7	277.3	269.9	284.5	352.1	290.3	261.0	246.8	287.6	291.9	276.1	272.7 B
N + Mo			255.8	275.0	265.5	285.3	351.3	286.7	273.3	255.8	287.0	290.4	280.3	277.3 A
0			262.0	268.0	270.1	280.9	352.5	286.7	269.3	245.1	287.0	290.1	274.3	273.2
5			256.7	275.7	267.2	292.1	349.5	288.2	261.0	259.7	288.7	285.5	282.9	275.5
10			273.1	279.2	268.7	283.4	349.9	290.9	262.1	250.9	284.2	297.7	271.4	273.2
20			257.2	281.6	264.8	283.2	354.9	288.4	276.2	249.5	289.4	291.3	284.3	278.2
p_Nsoil_contrast			0.09*	0.15	0.58	0.74	0.94	0.59	0.85	0.04*	0.19	0.96	0.83	0.73
p_Mo			0.17	0.61	0.12	0.87	0.73	0.14	0.11	0.13	0.81	0.73	0.60	0.08*
p_Nrate			0.55	0.57	0.57	0.32	0.31	0.67	0.44	0.35	0.54	0.32	0.60	0.51
p_Nrate_Mo			0.85	0.47	0.48	0.44	0.34	0.79	0.74	0.69	0.68	0.55	0.89	0.74
p_site								<0.01*						<0.01*
p_Site_Mo								0.37						0.40
p_Site_Nrate								0.38						0.53
p_Site_Mo_Nrate								0.78						0.96

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters rmeans N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S5. Stalk diameter of the 5 sites and the annual average for the year 1 and 2.

Soil	N	Mo	Site 1	Site 2	Site 3	Site 4	Site 5	Year 1	Site 1	Site 2	Site 3	Site 4	Site 5	Year 2
kg t^{-1}	**kg ha^{-1}**	**g ha^{-1}**	**--------------------- Diameter - mm (Year 1) ---------------------**						**--------------------- Diameter - mm (Year 2) ---------------------**					
-	-	-	23.0	24.5	24.4	23.4	28.7	24.8	23.8	24.0	24.5	24.8	23.7	24.2
0.8	0	-	23.5	25.2	24.1	23.3	28.4	24.9	24.3	23.3	24.8	24.1	22.6	23.8
0.8	5	-	23.8	24.8	23.4	24.3	28.0	24.9	23.3	23.2	25.0	24.4	23.8	24.0
0.8	10	-	25.2	24.7	23.4	23.1	27.9	24.8	23.4	24.2	24.3	24.3	24.5	24.1
0.8	20	-	24.0	23.9	23.9	23.2	28.9	24.8	24.7	23.7	25.2	24.2	24.8	24.5
0.8	0	100	22.8	24.2	23.6	24.0	27.6	24.4	24.8	23.9	24.7	23.8	23.4	24.1
0.8	5	100	23.2	24.2	24.1	24.3	27.5	24.7	24.7	24.1	24.5	23.8	22.8	24.0
0.8	10	100	24.5	23.4	24.1	24.2	28.4	24.9	24.5	24.3	24.2	24.1	24.2	24.3
0.8	20	100	24.0	24.9	23.3	24.1	28.7	25.0	25.4	24.0	24.9	22.4	23.0	23.9
N - Mo			24.1	24.7	23.7	23.4 B	28.0	24.8	23.9 B	23.6	24.8	24.2	23.9	24.1
N + Mo			23.6	24.2	23.8	24.1 A	28.3	24.7	24.8 A	24.1	24.6	23.5	23.3	24.1
0			23.1	24.7	23.9	23.6	28.0	24.7	24.5	23.6	24.7	24.0	23.0	24.0
5			23.5	24.5	23.7	24.3	27.8	24.8	24.0	23.7	24.8	24.1	23.3	24.0
10			24.8	24.1	23.7	23.6	28.2	24.9	23.9	24.2	24.3	24.2	24.3	24.2
20			24.0	24.4	23.6	23.6	28.8	24.9	25.0	23.8	25.0	23.3	23.9	24.2
p_Nsoil_contrast			0.53	0.34	0.71	0.83	0.16	0.84	0.61	0.44	0.80	0.46	0.27	0.41
p_Mo			0.34	0.18	0.83	0.03*	0.46	0.61	0.05*	0.14	0.33	0.09	0.47	0.91
p_Nrate			0.17	0.61	0.96	0.29	0.18	0.82	0.27	0.43	0.15	0.39	0.62	0.72
p_Nrate_Mo			0.95	0.12	0.54	0.52	0.52	0.55	0.89	0.82	0.89	0.43	0.68	0.45
p_site								<0.01*						<0.01*
p_Site_Mo								0.17						0.07*
p_Site_Nrate								0.81						0.44
p_Site_Mo_Nrate								0.62						0.96

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters means N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S6. SPAD index of the 5 sites and the annual average for the year 1 and 2.

Soil	**N**	**Mo**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 1**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 2**
kg t^{-1}	**kg ha^{-1}**	**g ha^{-1}**	**--------------------- SPAD (Year 1) ---------------------**						**--------------------- SAPD (Year 2) ---------------------**					
-	-	-	42.7	35.4	-	-	42.6	40.2	-	41.9	-	42.1	41.9	42.0
0.8	0	-	54.3	35.6	-	-	39.5 Bab	43.1	-	40.0	-	41.4	41.9	41.1
0.8	5	-	53.0	36.5	-	-	38.6 a	42.7	-	39.8	-	42.6	41.6	41.3
0.8	10	-	51.5	37.2	-	-	42.4 Aa	43.7	-	38.6	-	40.4	41.1	40.0
0.8	20	-	46.1	36.3	-	-	40.2 ab	40.8	-	38.9	-	41.3	42.3	40.8
0.8	0	100	42.0	37.1	-	-	43.7 Aa	40.9	-	40.2	-	40.0	42.3	40.8
0.8	5	100	47.8	34.5	-	-	37.2 bc	39.8	-	39.8	-	42.4	41.8	41.3
0.8	10	100	43.9	36.5	-	-	33.9 Bc	38.1	-	40.5	-	42.1	42.4	41.7
0.8	20	100	40.0	36.1	-	-	39.0 b	38.4	-	39.9	-	42.7	43.0	41.8
N - Mo			51.2 A	36.4	-	-	38.4	42.6 A	-	39.3	-	41.4	41.7	40.8 B
N + Mo			43.4 B	36.0	-	-	40.2	39.3 B	-	40.1	-	41.8	42.3	41.4 A
0			48.2	36.3	-	-	41.6	42.0	-	40.1	-	40.7	42.1	41.0
5			50.4	35.5	-	-	37.9	41.2	-	39.8	-	42.5	41.7	41.3
10			47.7	36.8	-	-	38.2	40.9	-	39.6	-	41.2	41.8	40.8
20			43.0	36.2	-	-	39.6	39.6	-	39.4	-	42.0	42.6	41.3
p_Nsoil_contrast			<0.01*	0.93	-	-	0.30	0.11	-	0.17	-	0.16	0.87	0.17
p_Mo			<0.01*	0.56	-	-	0.11	0.01*	-	0.14	-	0.59	0.22	0.07*
p_Nrate			0.12	0.47	-	-	0.08*	0.21	-	0.78	-	0.22	0.54	0.59
p_Nrate_Mo			0.65	0.24	-	-	0.01*	0.17	-	0.53	-	0.29	0.89	0.13
p_site								<0.01*						<0.01*
p_Site_Mo								<0.01*						0.84
p_Site_Nrate								0.03*						0.32
p_Site_Mo_Nrate								0.15						0.93

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters rmeans N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S7. Nitrogen foliar content of the 5 sites and the annual average for the year 1 and 2.

Soil	N	Mo	Site 1	Site 2	Site 3	Site 4	Site 5	Year 1	Site 1	Site 2	Site 3	Site 4	Site 5	Year 2
kg t^{-1}	kg ha^{-1}	g ha^{-1}	--------------------- g kg^{-1} N (Year 1) ---------------------						--------------------- g kg^{-1} N (Year 2) ---------------------					
-	-	-	15.9	16.9	17.8	17.3	18.7	17.3	-	18.3	17.2	-	18.8	18.1
0.8	0	-	15.3	17.3	17.7	19.5 Aa	17.0	17.4 ab	-	17.8	18.2	-	18.3	18.1
0.8	5	-	16.0	16.9	17.6	18.7 a	19.3	17.7 ab	-	18.5	17.9	-	19.3	18.6
0.8	10	-	16.1	16.6	17.7	18.8 a	21.1	18.3 a	-	17.9	17.3	-	19.8	18.3
0.8	20	-	15.2	14.5	18.4	16.4 b	20.0	16.9 Bb	-	18.1	18.3	-	20.3	18.9
0.8	0	100	14.8	17.3	18.3	17.2 B	19.5	17.4	-	18.4	17.8	-	18.7	18.3
0.8	5	100	15.3	16.4	17.6	17.4	19.3	17.2	-	19.0	17.8	-	19.0	18.6
0.8	10	100	16.0	16.5	18.6	17.4	19.2	17.5	-	18.8	17.9	-	19.4	18.7
0.8	20	100	16.6	16.7	18.8	17.6	20.0	17.9 A	-	18.1	17.9	-	19.5	18.5
N - Mo			15.6	16.3	17.8	18.4	19.3	17.5	-	18.1	17.9	-	19.4	18.5
N + Mo			15.7	16.7	18.3	17.4	19.5	17.5	-	18.6	17.9	-	19.2	18.5
0			15.1	17.3	18.0	18.4	18.3	17.4	-	18.1	18.0	-	18.5 b	18.2
5			15.6	16.7	17.6	18.0	19.3	17.4	-	18.7	17.9	-	19.2 ab	18.6
10			16.0	16.6	18.1	18.1	20.1	17.9	-	18.4	17.6	-	19.6 a	18.5
20			15.9	15.6	18.6	17.0	20.0	17.4	-	18.1	18.1	-	19.9 a	18.7
p_Nsoil_contrast			0.58	0.71	0.98	0.05*	0.46	0.26	-	0.44	0.17	-	0.48	0.94
p_Mo			0.83	0.45	0.26	0.03*	0.8	0.93	-	0.17	0.65	-	0.46	0.76
p_Nrate			0.28	0.14	0.43	0.13	0.3	0.62	-	0.57	0.30	-	0.06*	0.25
p_Nrate_Mo			0.23	0.23	0.23	0.06*	0.2	0.09*	-	0.89	0.22	-	0.71	0.50
p_site								<0.01*						<0.01*
p_Site_Mo								0.26						0.18
p_Site_Nrate								0.07*						0.09*
p_Site_Mo_Nrate								0.41						0.92

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters rmeans N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S8. Foliar P content of the 5 sites and the annual average for the year 1 and 2.

Soil	N	Mo	Site 1	Site 2	Site 3	Site 4	Site 5	Year 1	Site 1	Site 2	Site 3	Site 4	Site 5	Year 2
kg t^{-1}	**kg ha^{-1}**	**g ha^{-1}**	**--------------------- g kg^{-1} P (Year 1) ---------------------**						**--------------------- g kg^{-1} P (Year 2) ---------------------**					
-	-	-	2.0	2.6	1.9	2.5	1.8	2.1	-	1.7	1.5	-	1.5	1.6
0.8	0	-	1.8 Aa	2.3	1.9	2.3	2.4	2.1	-	1.5	1.6	-	1.4 B	1.5
0.8	5	-	1.7 ab	2.3	1.9	2.3	2.1	2.1	-	1.6	1.6	-	1.5	1.6
0.8	10	-	1.5 b	2.1	1.9	2.4	1.9	1.9	-	1.6	1.6	-	1.5	1.6
0.8	20	-	1.5 b	2.3	1.9	2.3	1.8	1.9	-	1.7	1.6	-	1.5	1.6
0.8	0	100	1.5 B	2.2	1.9	2.3	2.2	2.0	-	1.7	1.6	-	1.6 Aa	1.6
0.8	5	100	1.7	2.1	1.8	2.4	2.1	2.0	-	1.6	1.7	-	1.6 Aa	1.6
0.8	10	100	1.6	2.3	1.8	2.5	2.0	2.0	-	1.6	1.6	-	1.4 b	1.6
0.8	20	100	1.6	2.1	1.8	2.4	2.0	2.0	-	1.7	1.6	-	1.4 b	1.6
N - Mo			1.6	2.2	1.9 A	2.3	2.0	2.0	-	1.6	1.6	-	1.5	1.6
N + Mo			1.6	2.2	1.8 B	2.4	2.0	2.0	-	1.6	1.6	-	1.5	1.6
0			1.7	2.2	1.9	2.3	2.3 a	2.1	-	1.6	1.6	-	1.5	1.6
5			1.7	2.2	1.9	2.4	2.1 b	2.0	-	1.6	1.7	-	1.5	1.6
10			1.5	2.2	1.8	2.4	1.9 c	2.0	-	1.6	1.6	-	1.5	1.6
20			1.5	2.2	1.9	2.3	1.9 c	2.0	-	1.7	1.6	-	1.5	1.6
p_Nsoil_contrast			0.27	0.06*	0.75	0.35	0.03	0.53	-	0.12	0.99	-	0.35	0.15
p_Mo			0.71	0.28	0.01*	0.16	0.62	0.31	-	0.21	0.71	-	0.90	0.33
p_Nrate			0.27	0.92	0.26	0.56	<0.01*	<0.01*	-	0.18	0.46	-	0.54	0.33
p_Nrate_Mo			0.06*	0.21	0.48	0.96	0.11	0.48	-	0.31	0.66	-	0.01*	<0.01*
p_site								<0.01*						<0.01*
p_Site_Mo								0.23						0.75
p_Site_Nrate								0.03*						0.40
p_Site_Mo_Nrate								0.04*						0.34

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters rmeans N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S9. Potassium foliar content of the 5 sites and the annual average for the year 1 and 2.

Soil	N	Mo	Site 1	Site 2	Site 3	Site 4	Site 5	Year 1	Site 1	Site 2	Site 3	Site 4	Site 5	Year 2
kg t^{-1}	kg ha^{-1}	g ha^{-1}	--------------------- g kg^{-1} K (Year 1) ---------------------						--------------------- g kg^{-1} K (Year 2) ---------------------					
-	-	-	13.1	17.6	11.7	11.7	13.3	13.5	-	10.4	9.5	-	10.5	10.1
0.8	0	-	11.3	17.4	11.8	11.9	14.0	13.2	-	9.9	9.4	-	10.4	9.9
0.8	5	-	9.9	16.6	11.8	12.4	12.9	12.7	-	9.4	9.3	-	10.6	9.8
0.8	10	-	10.8	15.9	11.5	12.0	10.4	12.1	-	9.8	9.7	-	10.3	9.9
0.8	20	-	10.6	16.8	12.1	10.8	11.0	12.3	-	9.9	9.7	-	10.8	10.1
0.8	0	100	10.4	17.5	11.8	11.7	12.9	12.8	-	9.6	9.0	-	10.9	9.9
0.8	5	100	10.8	16.1	11.1	11.9	13.8	12.7	-	9.7	9.5	-	10.6	10.0
0.8	10	100	10.9	16.8	10.8	12.0	12.3	12.5	-	9.3	9.1	-	9.4	9.3
0.8	20	100	10.9	15.3	11.1	12.5	11.7	12.3	-	10.0	9.2	-	10.3	9.8
N - Mo			10.6	16.7	11.8 A	11.8	12.0	12.6	-	9.7	9.5	-	10.5	9.9
N + Mo			10.7	16.4	11.2 B	12.0	12.7	12.6	-	9.7	9.2	-	10.3	9.7
0			10.8	17.4	11.8 a	11.8	13.4 a	13.0 a	-	9.8	9.2	-	10.7	9.9
5			10.4	16.4	11.4 ab	12.2	13.3 a	12.7 ab	-	9.6	9.4	-	10.6	9.9
10			10.8	16.4	11.1 c	12.0	11.3 b	12.3 b	-	9.5	9.4	-	9.9	9.6
20			10.8	16.0	11.6 a	11.7	11.3 b	12.3 b	-	9.9	9.4	-	10.5	10.0
p_Nsoil_contrast			0.07*	0.86	0.92	0.91	0.70	0.33	-	0.32	0.74	-	0.88	0.40
p_Mo			0.86	0.51	<0.01*	0.46	0.21	0.98	-	0.67	0.28	-	0.32	0.20
p_Nrate			0.86	0.14	0.09*	0.76	<0.01*	0.06*	-	0.85	0.89	-	0.12	0.35
p_Nrate_Mo			0.57	0.26	0.21	0.13	0.22	0.87	-	0.70	0.76	-	0.23	0.27
p_site								<0.01*						<0.01*
p_Site_Mo								0.37						0.79
p_Site_Nrate								0.02*						0.56
p_Site_Mo_Nrate								0.08*						0.85

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters means N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S10. Calcium foliar content of the 5 sites and the annual average for the year 1 and 2.

Soil	**N**	**Mo**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 1**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 2**
kg t^{-1}	**kg ha^{-1}**	**g ha^{-1}**	**--------------------- g kg^{-1} Ca (Year 1) ---------------------**						**--------------------- g kg^{-1} Ca (Year 2) ---------------------**					
-	-	-	4.3	3.4	3.1	4.4	3.4	3.7	-	2.1	3.1	-	2.6	2.6
0.8	0	-	3.9	2.3	3.3	4.3	4.6 a	3.7	-	1.8	3.0	-	2.7	2.5
0.8	5	-	3.5	2.9	3.3	4.5	3.8 b	3.6	-	1.8	3.2	-	2.7	2.6
0.8	10	-	3.3	2.6	3.2	4.2	3.6 b	3.5	-	1.8	3.1	-	2.6	2.5
0.8	20	-	3.3	3.0	3.1	4.1	3.3 Bb	3.4	-	1.8	3.2	-	2.9	2.6
0.8	0	100	3.5	2.4	3.2	4.1	4.1	3.5	-	1.9	3.2	-	2.8	2.6
0.8	5	100	3.7	2.5	3.1	4.6	3.8	3.5	-	1.9	3.0	-	2.8	2.6
0.8	10	100	4.0	2.7	3.4	5.1	4.1	3.8	-	2.0	3.1	-	2.4	2.5
0.8	20	100	3.2	2.2	3.0	4.6	4.2 A	3.4	-	2.0	3.2	-	2.5	2.5
N - Mo			3.5	2.7	3.2	4.2 B	3.8	3.5	-	1.8 B	3.1	-	2.7	2.5
N + Mo			3.6	2.4	3.2	4.6 A	4.0	3.6	-	2.0 A	3.1	-	2.6	2.6
0			3.7	2.4	3.2	4.2	4.4	3.6	-	1.9	3.1	-	2.7	2.6
5			3.6	2.7	3.2	4.5	3.8	3.5	-	1.9	3.1	-	2.8	2.6
10			3.6	2.7	3.3	4.6	3.8	3.7	-	1.9	3.1	-	2.5	2.5
20			3.2	2.6	3.0	4.3	3.8	3.4	-	1.9	3.2	-	2.7	2.6
p_Nsoil_contrast			0.44	0.06*	0.75	0.79	0.23	0.52	-	0.13	0.49	-	0.78	0.26
p_Mo			0.51	0.28	0.62	0.05*	0.24	0.59	-	0.03*	0.97	-	0.29	0.71
p_Nrate			0.24	0.81	0.41	0.24	0.05*	0.56	-	0.97	0.87	-	0.28	0.66
p_Nrate_Mo			0.25	0.54	0.60	0.11	0.05*	0.23	-	0.91	0.54	-	0.11	0.42
p_site								<0.01*						<0.01*
p_Site_Mo								0.12						0.10
p_Site_Nrate								0.28						0.60
p_Site_Mo_Nrate								0.17						0.21

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters means N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S11. Magnesium foliar content of the 5 sites and the annual average for the year 1 and 2.

Soil	N	Mo	Site 1	Site 2	Site 3	Site 4	Site 5	Year 1	Site 1	Site 2	Site 3	Site 4	Site 5	Year 2
kg t^{-1}	**kg ha^{-1}**	**g ha^{-1}**	**--------------------- g kg^{-1} Mg (Year 1) ---------------------**						**--------------------- g kg^{-1} (Year 2) ---------------------**					
-	-	-	1.8	2.0	1.2	1.6	1.7	1.7	-	1.4	1.4	-	1.2	1.3
0.8	0	-	1.5	1.8	1.2	1.7	2.3	1.7	-	1.2	1.4	-	1.2	1.2
0.8	5	-	1.4	1.9	1.3	1.8	1.8	1.6	-	1.1	1.5	-	1.1	1.2
0.8	10	-	1.4	1.8	1.3	1.9	1.9	1.7	-	1.1	1.5	-	1.3	1.3
0.8	20	-	1.3	2.0	1.3	1.7	1.5	1.6	-	1.1	1.4	-	1.2	1.2
0.8	0	100	1.3	1.8	1.3	1.7	2.1	1.6	-	1.2	1.5	-	1.3	1.3
0.8	5	100	1.5	1.8	1.2	1.8	1.8	1.6	-	1.1	1.4	-	1.3	1.3
0.8	10	100	1.5	2.0	1.3	2.0	1.9	1.7	-	1.2	1.4	-	1.1	1.2
0.8	20	100	1.4	1.8	1.3	1.8	1.8	1.6	-	1.3	1.5	-	1.1	1.3
N - Mo			1.4	1.9	1.3	1.8	1.9	1.6	-	1.1	1.4	-	1.2	1.2
N + Mo			1.4	1.9	1.3	1.8	1.9	1.6	-	1.2	1.5	-	1.2	1.3
0			1.4	1.8	1.3	1.7 b	2.2 a	1.7	-	1.2	1.4	-	1.2	1.3
5			1.4	1.8	1.2	1.8 ab	1.8 b	1.6	-	1.1	1.5	-	1.2	1.3
10			1.4	1.9	1.3	1.9 a	1.9 b	1.7	-	1.2	1.4	-	1.2	1.3
20			1.3	1.9	1.3	1.7 b	1.7 b	1.6	-	1.2	1.5	-	1.2	1.3
p_Nsoil_contrast			0.23	0.18	0.99	0.40	0.07*	0.95	-	0.35	0.70	-	0.99	0.33
p_Mo			0.71	0.95	0.99	0.66	0.95	0.83	-	0.17	0.51	-	0.54	0.16
p_Nrate			0.73	0.73	0.78	0.08*	0.02*	0.42	-	0.53	0.55	-	0.92	0.92
p_Nrate_Mo			0.21	0.31	0.78	0.70	0.44	0.58	-	0.43	0.21	-	0.11	0.44
p_site								<0.01*						<0.01*
p_Site_Mo								0.99						0.93
p_Site_Nrate								0.01*						0.79
p_Site_Mo_Nrate								0.35						0.03*

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters means N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S12. Sulfur foliar content of the 5 sites and the annual average for the year 1 and 2.

Soil	**N**	**Mo**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 1**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 2**
kg t^{-1}	**kg ha^{-1}**	**g ha^{-1}**	**--------------------- g kg^{-1} S (Year 1) ---------------------**						**--------------------- g kg^{-1} S (Year 2) ---------------------**					
-	-	-	1.8	1.2	1.7	2.1	1.4	1.6	-	1.1	1.1	-	1.2	1.1
0.8	0	-	1.6	1.2 b	1.5	2.1	1.4 Aa	1.6	-	1.0	1.2	-	1.2	1.1
0.8	5	-	1.6	1.4 Aa	1.5	2.3	1.2 b	1.6	-	1.0	1.2	-	1.3	1.1
0.8	10	-	1.7	1.0 Bc	1.6	2.3	1.0 Abc	1.6	-	1.0	1.2	-	1.2	1.1
0.8	20	-	1.7	1.3 ab	1.5	1.9	1.0 Bc	1.5	-	1.0	1.2	-	1.3	1.1
0.8	0	100	1.7	1.2 ab	1.5	2.1	1.2 B	1.5	-	1.0	1.2	-	1.3 ab	1.1
0.8	5	100	1.5	1.1 Bb	1.3	2.0	1.4	1.5	-	1.1	1.2	-	1.3 a	1.1
0.8	10	100	1.5	1.4 Aa	1.6	2.4	1.3 A	1.6	-	1.1	1.2	-	1.1 c	1.1
0.8	20	100	1.5	1.2 ab	1.4	2.3	1.3 A	1.5	-	1.1	1.2	-	1.2 bc	1.1
N - Mo			1.6	1.3	1.5	2.2	1.1	1.5	-	1.0 B	1.2	-	1.2	1.1
N + Mo			1.5	1.2	1.4	2.2	1.3	1.5	-	1.1 A	1.2	-	1.2	1.1
0			1.6	1.2	1.5	2.1	1.3	1.6	-	1.0	1.2	-	1.2	1.1
5			1.5	1.3	1.4	2.1	1.3	1.5	-	1.0	1.2	-	1.3	1.1
10			1.6	1.2	1.6	2.4	1.1	1.6	-	1.0	1.2	-	1.2	1.1
20			1.6	1.3	1.4	2.1	1.1	1.5	-	1.1	1.2	-	1.2	1.1
p_Nsoil_contrast			0.54	0.90	0.39	0.90	0.39	0.35	-	0.09*	0.05*	-	0.15	0.78
p_Mo			0.21	0.50	0.51	0.89	0.02*	0.12	-	0.01*	0.86	-	0.94	0.10
p_Nrate			0.82	0.56	0.36	0.47	0.07*	0.58	-	0.83	0.82	-	0.06*	0.35
p_Nrate_Mo			0.56	<0.01*	0.89	0.35	<0.01*	0.19	-	0.82	0.89	-	0.06*	0.33
p_site								<0.01*						<0.01*
p_Site_Mo								0.81						0.12
p_Site_Nrate								0.42						0.13
p_Site_Mo_Nrate								0.16						0.13

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters means N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S13. Iron foliar content of the 5 sites and the annual average for the year 1 and 2.

Soil	**N**	**Mo**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 1**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 2**
kg t^{-1}	**kg ha^{-1}**	**g ha^{-1}**	**--------------------- mg kg^{-1} Fe (Year 1) ---------------------**						**--------------------- mg kg^{-1} Fe (Year 2) ---------------------**					
-	-	-	63.4	82.6	70.7	66.4	68.8	70.4	-	42.5	72.6	-	55.4	56.8
0.8	0	-	57.2	82.6	71.7	68.5	90.6	74.1	-	40.2	75.8	-	55.2 b	58.0
0.8	5	-	51.7	84.3	65.2	75.9	72.8	70.0	-	40.8	79.0	-	55.4 b	59.9
0.8	10	-	56.7	68.9	67.0	76.7	69.4	68.5	-	40.2	82.0	-	56.0 Ab	61.1
0.8	20	-	50.5	79.7	71.8	67.4	65.7	67.0	-	40.8	83.1	-	60.3 Aa	62.0
0.8	0	100	55.7	76.2	72.9	66.7	88.2	71.9	-	41.9	79.3	-	55.2	58.8
0.8	5	100	56.3	76.7	65.2	77.3	78.5	70.8	-	41.6	75.1	-	55.5	57.4
0.8	10	100	52.8	75.8	68.7	72.8	73.9	68.8	-	43.4	83.3	-	51.7 B	63.4
0.8	20	100	53.6	63.8	67.0	76.3	78.4	67.8	-	43.2	87.3	-	52.3 B	65.3
N - Mo			54.0	78.8	68.9	72.1	74.6 A	69.9	-	40.5 B	80.0	-	56.7	60.2
N + Mo			54.6	73.1	68.4	73.3	79.7 B	69.8	-	42.5 A	81.3	-	53.7	61.2
0			56.4	79.4	72.3	67.6	89.4 A	73.0 a	-	41.0	77.5	-	55.2	57.9
5			54.0	80.5	65.2	76.6	75.6 b	70.4 ab	-	41.2	77.1	-	55.4	57.9
10			54.8	72.3	67.9	74.7	71.6 b	68.6 b	-	41.8	82.7	-	53.8	59.4
20			52.1	71.7	69.4	71.9	72.0 b	67.4 b	-	42.0	85.2	-	56.3	61.2
p_Nsoil_contrast			0.32	0.99	0.88	0.73	<0.01*	0.25	-	0.49	0.34	-	0.94	0.91
p_Mo			0.81	0.13	0.83	0.72	<0.01*	0.93	-	0.06*	0.59	-	0.01*	0.92
p_Nrate			0.64	0.23	0.18	0.22	<0.01*	0.05*	-	0.88	0.06*	-	0.48	0.05*
p_Nrate_Mo			0.58	0.21	0.73	0.49	0.29	0.85	-	0.86	0.61	-	0.05*	0.75
p_site								<0.01*						<0.01*
p_Site_Mo								0.28						0.06*
p_Site_Nrate								<0.01*						0.09*
p_Site_Mo_Nrate								0.23						0.31

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters means N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S14. Manganese foliar content of the 5 sites and the annual average for the year 1 and 2.

Soil	**N**	**Mo**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 1**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 2**
kg t^{-1}	**kg ha^{-1}**	**g ha^{-1}**	**-------------------- mg kg^{-1} Mn (Year 1) --------------------**						**-------------------- mg kg^{-1} Mn (Year 2) --------------------**					
-	-	-	38.5	135.8	48.2	47.3	48.0	63.5	-	78.8	37.6	-	39.3	51.9
0.8	0	-	28.5	128.7	41.6	53.0	59.9	62.4	-	74.1	33.4	-	40.4	49.3
0.8	5	-	27.2	121.7	43.4	51.0	45.4	57.7	-	73.3	37.7	-	35.3	48.8
0.8	10	-	31.5	118.4	40.9	56.6	42.9	58.6	-	87.0	35.0	-	40.7	54.2
0.8	20	-	29.9	137.0	40.8	47.2	39.3	58.8	-	74.7	31.9	-	36.3	47.7
0.8	0	100	32.0	129.5	38.6	45.3	53.3	59.8	-	81.0	32.0	-	40.6	51.2
0.8	5	100	32.4	112.1	39.7	48.3	40.6	54.6	-	78.4	31.8	-	37.5	49.2
0.8	10	100	34.6	134.2	38.7	57.5	55.6	64.1	-	81.2	37.1	-	30.6	49.6
0.8	20	100	26.1	117.8	39.4	51.4	50.3	57.0	-	76.6	34.4	-	36.1	49.0
N - Mo			29.3	126.5	41.7	51.9	49.9	59.4	-	77.3	34.5	-	38.2	50.0
N + Mo			31.3	123.4	39.1	50.6	46.8	58.9	-	79.3	33.8	-	36.2	49.8
0			30.3	129.1	40.1	49.2 b	56.6	61.1	-	77.6	32.7	-	40.5	50.2
5			29.8	116.9	41.5	49.7 b	43.0	56.2	-	75.9	34.7	-	36.4	49.0
10			33.0	126.3	39.8	57.0 a	49.2	61.4	-	84.1	36.0	-	35.6	51.9
20			28.0	127.4	40.1	49.3 b	44.8	57.9	-	75.7	33.1	-	36.2	48.3
p_Nsoil_contrast			0.28	0.44	0.48	0.53	0.57	0.54	-	0.48	0.53	-	0.87	0.49
p_Mo			0.32	0.71	0.19	0.56	0.51	0.85	-	0.69	0.69	-	0.45	0.88
p_Nrate			0.36	0.71	0.82	0.06*	0.19	0.22	-	0.34	0.50	-	0.56	0.41
p_Nrate_Mo			0.41	0.47	0.98	0.31	0.32	0.33	-	0.63	0.31	-	0.38	0.46
p_site								<0.01*						<0.01*
p_Site_Mo								0.91						0.58
p_Site_Nrate								0.72						0.43
p_Site_Mo_Nrate								0.43						0.57

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters means N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S15. Zinc foliar content of the 5 sites and the annual average for the year 1 and 2.

Soil	**N**	**Mo**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 1**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Year 2**
kg t^{-1}	**kg ha^{-1}**	**g ha^{-1}**	**--------------------- mg kg^{-1} Zn (Year 1) ---------------------**						**--------------------- mg kg^{-1} Zn (Year 2) ---------------------**					
-	-	-	4.3	5.4	3.9	3.9	4.5	4.4	-	6.2	4.2	-	4.4	4.9
0.8	0	-	3.7	5.5 a	3.8	3.6	4.6	4.3	-	5.8	4.3	-	4.0	4.7
0.8	5	-	3.1	5.6 Aa	4.0	3.7	3.9	4.1	-	6.0	4.3	-	4.2	4.9
0.8	10	-	3.4	4.8 Bb	3.9	3.6	3.5	3.9	-	5.9	4.4	-	4.2	4.8
0.8	20	-	3.1	5.3 ab	4.0	3.2	3.1	3.7	-	5.7	4.4	-	4.5	4.8
0.8	0	100	3.3	5.4 ab	3.9	3.2	4.3	4.0	-	6.1	4.4	-	4.5	5.0
0.8	5	100	3.3	4.8 Bb	3.7	3.7	3.7	3.8	-	6.1	4.3	-	4.5	4.9
0.8	10	100	3.3	5.5 Aab	3.6	4.1	3.5	4.0	-	5.9	4.5	-	3.9	4.8
0.8	20	100	3.4	4.8 b	3.7	3.8	3.7	3.9	-	5.7	4.5	-	4.2	4.8
N - Mo			3.3	5.3	3.9	3.5	3.8	4.0	-	5.8	4.3	-	4.2	4.8
N + Mo			3.3	5.1	3.7	3.7	3.8	3.9	-	5.9	4.4	-	4.3	4.9
0			3.5	5.4	3.9	3.4	4.5 a	4.1 a	-	5.9	4.4	-	4.2	4.8
5			3.2	5.2	3.8	3.7	3.8 b	3.9 ab	-	6.0	4.3	-	4.3	4.9
10			3.4	5.1	3.7	3.9	3.5 b	3.9 ab	-	5.9	4.5	-	4.1	4.8
20			3.2	5.0	3.8	3.5	3.4 b	3.8 b	-	5.7	4.5	-	4.3	4.8
p_Nsoil_contrast			0.12	0.85	0.85	0.54	0.59	0.22	-	0.07*	0.68	-	0.12	0.09*
p_Mo			0.84	0.45	0.12	0.28	0.95	0.73	-	0.50	0.29	-	0.82	0.28
p_Nrate			0.38	0.41	0.91	0.16	<0.01	0.06*	-	0.44	0.41	-	0.26	0.83
p_Nrate_Mo			0.21	0.04*	0.70	0.11	0.04	0.18	-	0.82	0.78	-	0.02	0.22
p_site								<0.01*						<0.01*
p_Site_Mo								0.57						0.86
p_Site_Nrate								0.01*						0.21
p_Site_Mo_Nrate								0.02*						0.46

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters means N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S16. Cuper foliar content of the 5 sites and the annual average for the year 1 and 2.

Soil	N	Mo	Site 1	Site 2	Site 3	Site 4	Site 5	Year 1	Site 1	Site 2	Site 3	Site 4	Site 5	Year 2
kg t^{-1}	kg ha^{-1}	g ha^{-1}	-------------------- mg kg^{-1} Cu (Year 1) --------------------						-------------------- mg kg^{-1} Cu (Year 2) --------------------					
-	-	-	17.8	19.5	14.9	16.6	15.6	16.9	-	16.9	10.5	-	10.1	12.5
0.8	0	-	11.3	23.5	11.1	12.4	13.0 ab	14.2	-	16.1	11.3	-	9.6 B	12.3
0.8	5	-	8.7	16.6	13.7	17.1	10.0 Bb	13.2	-	16.2	11.9	-	8.8 B	14.1
0.8	10	-	10.1	18.5	13.2	16.1	15.8 Aa	15.7	-	17.3	11.5	-	9.6	12.8
0.8	20	-	10.7	21.5	13.7	16.8	10.2 Bb	14.6	-	16.4	11.6	-	10.1	12.7
0.8	0	100	10.0	25.3	10.1	17.2	10.9 ab	14.7	-	16.3	11.1	-	11.6 Aa	13.7
0.8	5	100	9.4	17.8	12.9	18.2	14.9 Aa	14.6	-	16.4	11.3	-	11.1 Aab	13.8
0.8	10	100	9.9	18.1	13.8	17.5	10.1 Bb	13.9	-	17.1	11.1	-	9.5 c	14.1
0.8	20	100	12.1	23.4	13.5	16.3	14.8 Aa	16.0	-	16.7	11.3	-	10.3 bc	14.0
N - Mo			10.2	20.0	12.9	15.6	12.7	14.4	-	16.5	11.6	-	9.5	13.3
N + Mo			10.3	21.1	12.6	17.3	12.2	14.8	-	16.6	11.2	-	10.6	13.9
0			10.6	24.4 A	10.6 b	14.8	12.0	14.5	-	16.2	11.2	-	10.6	13.7
5			9.1	17.2 B	13.3 a	17.6	12.4	13.9	-	16.3	11.6	-	10.0	14.0
10			10.0	18.3 B	13.5 a	16.8	12.9	14.8	-	17.2	11.3	-	9.6	13.4
20			11.4	22.4 AB	13.6 a	16.5	12.5	15.3	-	16.5	11.5	-	10.2	13.3
p_Nsoil_contrast			0.03*	0.19	0.20	0.15	0.79	0.08*	-	0.28	0.29	-	0.55	0.73
p_Mo			0.87	0.60	0.67	0.13	0.74	0.28	-	0.79	0.16	-	<0.01*	0.20
p_Nrate			0.43	0.09*	0.05*	0.29	0.96	0.79	-	0.37	0.74	-	0.27	0.98
p_Nrate_Mo			0.81	0.98	0.91	0.36	0.02*	0.28	-	0.97	0.96	-	0.07*	0.41
p_site								<0.01*						<0.01*
p_Site_Mo								0.75						0.02*
p_Site_Nrate								0.03*						0.18
p_Site_Mo_Nrate								0.47						0.54

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters means N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S17. Boron foliar content of the 5 sites and the annual average for the year 1 and 2.

Soil	N	Mo	Site 1	Site 2	Site 3	Site 4	Site 5	Year 1	Site 1	Site 2	Site 3	Site 4	Site 5	Year 2
kg t^{-1}	**kg ha^{-1}**	**g ha^{-1}**	**--------------------- mg kg^{-1} B (Year 1) ---------------------**						**--------------------- mg kg^{-1} B (Year 2) ---------------------**					
-	-	-	7.1	14.7	7.4	12.8	14.9	11.4	-	7.8	2.1	-	3.1	4.3
0.8	0	-	2.5	13.5	7.8	11.5	14.2	10.0	-	7.7	1.1	-	2.6	3.8
0.8	5	-	2.1	13.4	8.0	11.7	12.9	9.6	-	8.6	0.8	-	2.9	4.1
0.8	10	-	2.0	13.0	8.1	12.1	10.7	9.5	-	8.8	1.9	-	3.4	4.7
0.8	20	-	2.7	13.2	10.5	11.7	8.9	9.4	-	7.1	1.5	-	3.2	3.9
0.8	0	100	2.5	14.5	8.1	10.6	13.9	9.9	-	8.3	3.0	-	6.5	5.9
0.8	5	100	2.5	12.1	9.1	9.9	10.2	9.1	-	7.1	3.9	-	7.0	6.0
0.8	10	100	3.6	16.9	8.3	12.7	9.8	10.2	-	7.8	3.8	-	5.4	5.7
0.8	20	100	2.3	15.0	9.2	9.2	11.3	9.4	-	7.3	3.6	-	5.2	5.4
N - Mo			2.3	13.2	8.7	11.8 A	11.3	9.6	-	8.0	1.3 B	-	3.0 B	4.1 B
N + Mo			2.7	14.6	8.7	10.6 B	11.7	9.7	-	7.6	3.5 A	-	6.0 A	5.7 A
0			2.5	14.0	7.9	11.0	14.1	10.0	-	8.0	2.0	-	4.5	4.8
5			2.3	12.7	8.6	10.8	11.6	9.4	-	7.8	2.3	-	4.9	5.0
10			2.8	15.0	8.2	12.4	10.2	9.9	-	8.3	2.8	-	4.4	5.2
20			2.5	14.1	9.9	10.5	10.1	9.4	-	7.2	2.5	-	4.2	4.6
p_Nsoil_contrast			<0.01*	0.46	0.39	0.43	0.57	0.01*	-	0.81	0.13	-	0.44	0.15
p_Mo			0.45	0.29	0.96	0.05*	0.75	0.62	-	0.28	<0.01*	-	<0.01*	<0.01*
p_Nrate			0.92	0.68	<0.01	0.12	0.11	0.56	-	0.13	0.65	-	0.65	0.25
p_Nrate_Mo			0.53	0.58	0.17	0.27	0.52	0.21	-	0.13	0.14	-	0.14	0.18
p_site								<0.01*						<0.01*
p_Site_Mo								0.36						<0.01*
p_Site_Nrate								0.09*						0.35
p_Site_Mo_Nrate								0.81						0.09*

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters means N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

Table S18. Molybdenum foliar content of the 5 sites and the annual average for the year 1 and 2.

Soil	N	Mo	Site 1	Site 2	Site 3	Site 4	Site 5	Year 1	Site 1	Site 2	Site 3	Site 4	Site 5	Year 2
kg t^{-1}	kg ha^{-1}	g ha^{-1}	---------------------- mg kg^{-1} Mo (Year 1) ----------------------						---------------------- mg kg^{-1} Mo (Year 2) ----------------------					
-	-	-	0.8	0.6	1.0	0.6	0.6	0.7	-	0.3	0.3	-	0.8	0.4
0.8	0	-	0.7	0.7	0.7 a	0.5	0.9	0.6	-	0.2 B	0.3	-	0.6	0.4
0.8	5	-	0.6	0.6	0.4 ab	0.6	0.8	0.6	-	0.3 B	0.2	-	0.6	0.4
0.8	10	-	0.4	0.5	0.4 Bc	1.0	0.8	0.5	-	0.3 B	0.2	-	0.5	0.4
0.8	20	-	0.7	0.8	0.6 Bab	1.0	0.7	0.7	-	0.2 B	0.3	-	0.5	0.4
0.8	0	100	1.3	1.3	0.7 bc	0.8	0.4	1.0	-	0.6 Ac	0.6	-	0.9	0.8
0.8	5	100	1.4	1.1	0.4 c	1.0	0.5	0.9	-	0.6 Abc	0.6	-	0.9	0.8
0.8	10	100	1.5	1.3	1.1 Aa	1.6	0.3	1.2	-	0.9 Aab	0.6	-	0.9	0.7
0.8	20	100	1.8	0.8	1.0 Aab	1.4	0.5	1.1	-	1.1 Aa	0.6	-	1.1	0.8
N - Mo			0.6 B	0.7 B	0.5	0.7 B	0.4 B	0.6 B	-	0.3	0.3 B	-	0.5 B	0.4 B
N + Mo			1.5 A	1.2 A	0.8	1.2 A	0.7 A	1.1 A	-	0.8	0.6 A	-	0.9 A	0.8 A
0			1.0	1.0	0.7	0.7 c	0.6	0.8	-	0.4	0.4	-	0.7	0.6
5			1.0	0.9	0.4	0.8 bc	0.7	0.7	-	0.5	0.4	-	0.8	0.6
10			1.0	0.9	0.7	1.3 a	0.5	0.9	-	0.6	0.4	-	0.7	0.5
20			1.2	0.8	0.8	1.2 ab	0.6	0.9	-	0.7	0.5	-	0.8	0.6
p_Nsoil_contrast			0.98	0.67	0.34	0.62	0.37	0.94	-	0.83	0.99	-	0.05*	0.28
p_Mo			<0.01*	0.05*	0.01*	0.01*	<0.01*	<0.01*	-	<0.01*	<0.01*	-	<0.01*	<0.01*
p_Nrate			0.65	0.92	0.06*	0.05*	0.68	0.28	-	0.17	0.82	-	0.54	0.14
p_Nrate_Mo			0.64	0.67	0.02*	0.95	0.44	0.19	-	0.04*	0.79	-	0.59	0.10
p_site								<0.01*						<0.01*
p_Site_Mo								<0.01*						0.03*
p_Site_Nrate								0.31						0.45
p_Site_Mo_Nrate								0.86						0.07*

p_Nsoil_contrast was evaluated by the t-test (p<.1) between the plot with and without N fertilization in the soil without foliar spray. The factors and interactions were evaluated by the LSD test (p<.1). Uppercase letters means Mo application differences, lowercase letters means N rates differences. Due to operational difficulties samples from site 1 and 4 leaf were not collected in the second year.

4. FINAL CONSIDERATION

The focus of this study was on the factors that decision-makers need to consider before applying foliar fertilizers for sugarcane crop. The research primarily (chapter 1) examined the plant nutrient status, with a specific emphasis on nutrient availability. The data revealed that the best N concentration in nutrient solution for sugarcane growth is 3 mM N, which represents only 20% of the N concentration in the original Hoagland and Arnon (1950) solution.

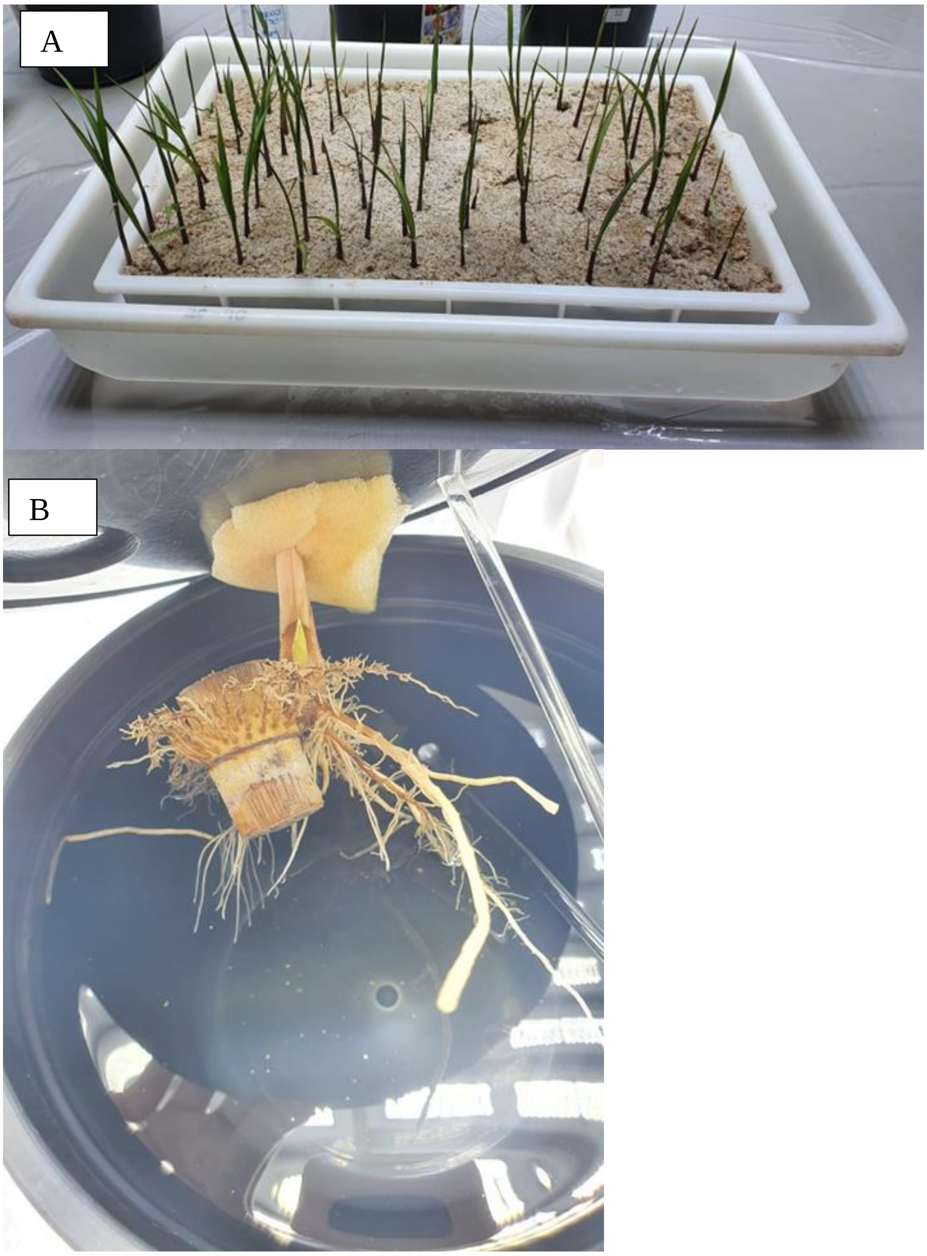
It was observed that plants with excess N experienced an impaired roots-to-shoot ratio. In contrast, plants supplied with a 20% N solution, which resulted in the best plant development, demonstrated a root:shoot ratio of 1:1.3. On the other hand, N-deficient plants had a ratio of 1:1.6, while plants with N-excess exhibited a ratio of 1:0.5. This finding provided insights into the lack of response of sugarcane to high N rates, suggesting that an excessive amount of N supplied early in the growth stage might lead to a larger shoot than the root system can support.

The chapter 2 highlighted that N-deficient plants showed higher remobilization of N and Mo administered through the leaves. Furthermore, $N\text{-}NH_4$ levels in the roots of deficient plants increased significantly. This suggests that N-deficient plants should prioritize addressing major nutritional deficits in the roots before investing in new shoots. Moreover, when isolated Mo was administered to well-nourished plants, a reduction in nitrate levels in the top leaves was observed. This indicates that N-adequate plants make better use of foliar Mo applied. Furthermore, the recovery of applied N varied depending on its availability in the system. Well-nourished plants demonstrated a greater capability to absorb and allocate this nutrient compared to N-deficient plants. The study revealed that foliar fertilization with N and Mo yielded the highest returns in well-nourished plants.

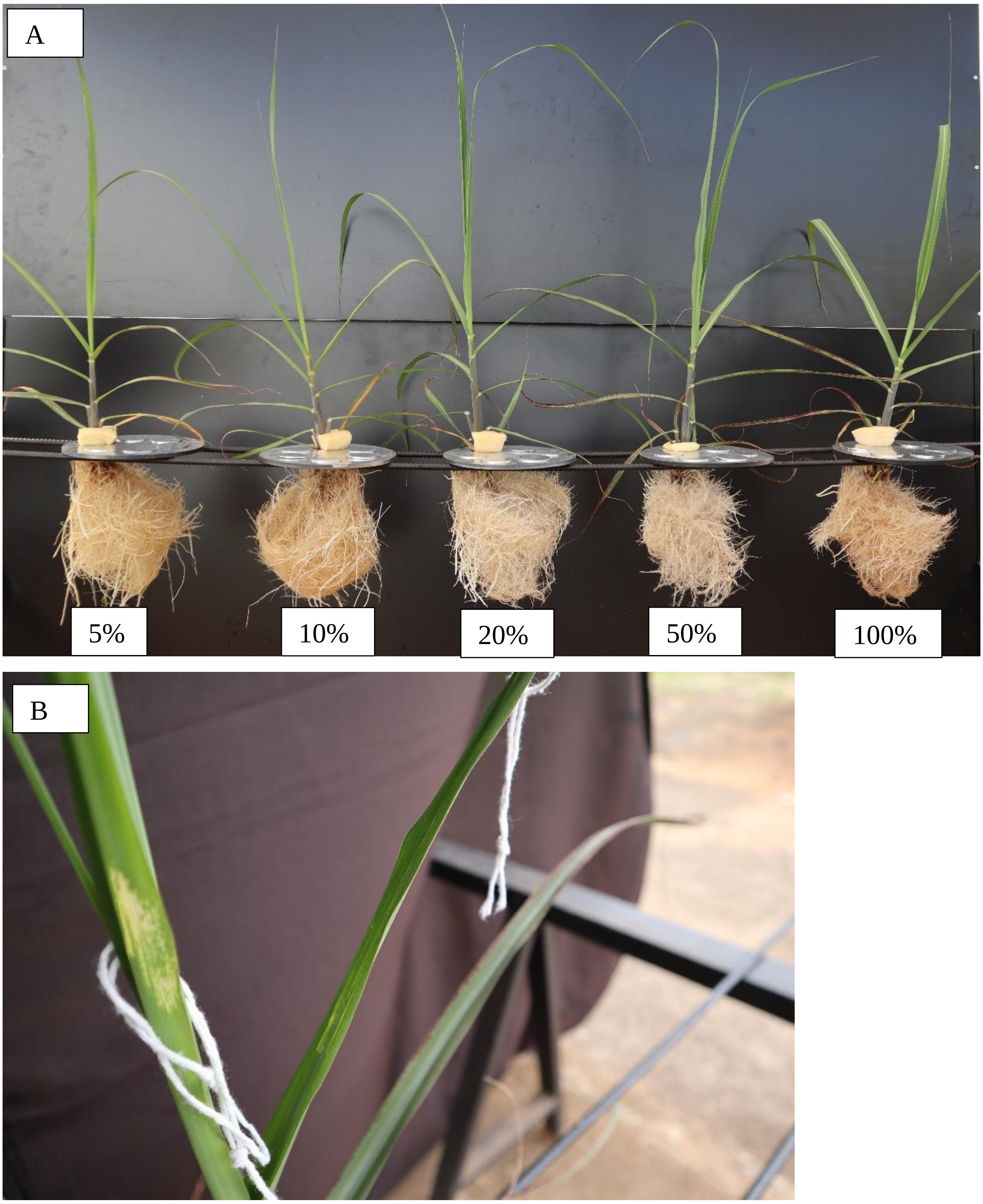
The chapter 3, developed under five field conditions during two crop seasons (2017/2018 and 2018/2019), demonstrated that the optimal N rate for foliar fertilization in sugarcane is 5 kg ha^{-1} N. It was observed that applying this rate of N through foliar applications resulted in yield gains, even in years with limited rainfall. However, caution must be taken with N rates of 10 and 20 kg ha^{-1} N particularly in years with significant water deficits following application. These higher N rates can have deleterious effects on sugarcane yield, especially under water-restricted conditions. Therefore, it is crucial to adopt strict criteria regarding environmental conditions when implementing foliar fertilization practices. Additionally, the research highlights the importance of Mo in sugarcane maturation and senescence, with late-season applications of Mo playing a significant role. By incorporating these findings into sugarcane management practices, farmers can optimize foliar fertilization strategies and enhance crop performance. Further research can explore the underlying mechanisms of Mo in sugarcane maturation process, contributing to improved agricultural practices.

APPENDIX

Supplementary figures from section 1



Supplementary figures 1. Seedlings germination in the washed sand (A); and plants support detail (B).



Supplementary figures 2. Injured leaves due to the urea application (A); and plants without N foliar N treatment in the N supplies (B).

Supplementary figures from section 2

Supplementary figures 3. Seedling selection (A); and pot details with gray paint and Styrofoam support (B).

Supplementary figures from section 3

Supplementary figures 4. Plot installation with based fertilization (A); Foliar treatment application (B); and leave sampling (C).