ISSN 0100-2945

DOI: http://dx.doi.org /10.1590/0100-29452021163

Revista Brasileira de
Fruticultura

Soils and plant nutrition

Does molybdenum and cobalt foliar spray mitigate nitrate starvation and ammonium deprivation stress?

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Abstract - Knowledge about the nutritional balance at the initial phenological stage is mandatory to overcome limitations on nutritional availabilities required by the plant species. However, little is elucidated about nitrate (NO_3^-) and ammonium (NH_4^+) deprivation stress. Our hypothesis tested is that there are benefits of the foliar application (spray) of molybdenum (Mo) and cobalt (Co) under different availabilities (ionic strength, IS) from the presence of nitric sources ($CaNO_3^{2-}$) and absence of ammonium ($NH_4H_2PO_4$) in root application (hydroponic cultivation) at the initial phenological growth stage. Nutrient supply was carried out with a nutrient solution, which is deprived of NH_4^+. Treatments were 25%, 50%, and 100% IS, supplied via hydroponic cultivation, combined with the absence/presence of Co/Mo spray. Plants were randomly distributed into 17 blocks (replicates) with 6 treatments conducted in a factorial scheme and data were analyzed by ANOVA and ANCOVA. We observed that Co/Mo spray diminished plant growth discrepancies between treatments at different IS's. In addition, contents of photosynthetic pigments were higher at 25% IS without Co/Mo spray. Thus, we concluded that Mo and Co spray can alleviate NO_3^- starvation/NH_4^+ deprivation stress during the initial growth phenological stages of yellow passion fruits.

Index terms: abiotic stress; beneficial mineral elements; essential mineral elements; hydroponic; passiflora; plant nutrition.

Aplicação foliar de cobalto e molibdênio mitiga a privação de nitrato e ausência de amônio?

Resumo - O conhecimento do balanço nutricional na fase fenológica inicial é crucial para contornar as limitações das disponibilidades nutricionais exigidas por espécies vegetais. No entanto, pouco é sabido sobre o estresse de privação de nitrato (NO_3^-) e ausência de amônio (NH_4^+). Nossa hipótese testou se há benefícios da aplicação foliar (*spray*) de molibdênio (Mo) e cobalto (Co) em diferentes disponibilidades (força iônica, IS), a partir da presença restrita de N em fontes nítricas ($CaNO_3^{2-}$) e ausência de N em formas amoniacais ($NH_4H_2PO_4$), por aplicação radicular (cultivo hidropônico), na fase fenológica de crescimento inicial. Nutrientes foram fornecidos usando solução nutritiva, desprovida de NH_4^+. Os tratamentos empregados foram 25%, 50% e 100% IS, fornecidos via cultivo hidropônico, combinados com ausência / presença de pulverização de Co/Mo. As plantas foram distribuídas ao acaso, em 17 blocos (repetições), com 6 tratamentos conduzidos em esquema fatorial, e os dados foram analisados por ANOVA e ANCOVA. Observou-se que a pulverização de Co/Mo diminuiu as discrepâncias entre o crescimento da planta em diferentes ISs. Além disso, os conteúdos de pigmentos fotossintéticos foram maiores que 25% IS sem pulverização de Co/Mo. Assim, concluiu-se que a pulverização foliar de Mo e Co pode aliviar o estresse de privação de NO_3^- /ausência de NH_4^+ durante os estágios fenológicos de crescimento inicial do maracujá-amarelo.

Termos para indexação: estresse abiótico; elementos minerais benéficos; elementos minerais essenciais; hidroponia; passiflora; nutrição vegetal.

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Received: December 15, 2020
Accepted: April 12, 2021

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Introduction

Fresh yellow passion fruits [*Passiflora edulis* Sims. f. *flavicarpa* Degener] and derivatives are sources of antioxidants beneficial to human health, which makes this species an economic alternative for small farmers (OLUOCH; NYABOGA; BARGUL, 2018). Commercial orchards with high productivity and economic profitability comes from the use of seedlings with excellent vigor and nutritionally balanced. It is crucial to plants adapted to the constant environmental changes, for example, nitrogen (N) forms of starvation and/or deprivation in the soil (WANG; MIAO; LI, 2015).

Ammonium (NH_4^+) and nitrate (NO_3^-) are major nitrogen (N) forms taken up by plants, and amounts consist of 50% above total ions (YIN; LUO; WANG; SHEN *et al.*, 2014). Plants have different NH_4^+ uptake preferences, but to seedlings, it is poorly understood. At initial phenological growth stages, literature reported that these two forms were both effective N sources for the direct absorption of plants and several hydroponic experiments have investigated of NH_4^+ and NO_3^- uptake (HUANG; LI; ZHOU; SUN *et al.*, 2018).

According to Zeng and colleagues (ZENG; XU; WU; HUANG et al., 2014) plants grown in nutrient solution with lower N availability have lower total chlorophyll concentrations, since this nutrient is an integral part of photosynthetic pigments, Ribulose 1,5-Bisphosphate Carboxylase/Oxygenase (RuBisCO) (ESTRADA; BÖHLKE; STURCHIO; GU et al., 2017) however, there is no single nutrient solution common to all plant species and/or phonological stages.

Molybdenum (Mo) is beneficial for leaf application in young strawberry plants (*Fragaria x ananassa* Duch. cv. *Akihime*), since it improves NO_3^- transport by roots and increasing the efficient use of N by plants (LIU; BURGOS; ZHANG; TANG et al., 2017). This essential mineral promotes the enzymatic catalysis of a series of biochemical reactions, for example, synthesis of phytohormones, sulfite detoxification (HIPPLER; BOARETTO; DOVIS; GOMES et al., 2017). The transport of nitrogen compounds essential in eukaryotic organisms, such as molybdoenzymes, bind to Pterine ($C_6H_5N_5O$) to form the MoCo cofactor, and a constituent of the nitrate reductase enzyme (CHATTERJEE; BANDYOPADHYAY, 2017; HÄNSCH; MENDEL, 2009). In this way, the higher the nitrate reductase activity, the lower the nitrate content in the cytoplasm of plant cells.

Cobalt (Co) is considered a beneficial mineral element for plants, used as cofactor of various enzymes and proteins; however, scientific reports have pointed to irreversible damage in barley genotypes (*Hordeum vulgare* L.), such as reduction in cell division, vein discoloration, leaf abscission, abnormal translocation of root-to-leaf nutrients and chloroplast integrity and iron-porphyrin enzyme disorders (LANGE; FAUCON; DELHAYE; HAMITI et al., 2017; SHAHID; DUMAT; KHALID; SCHRECK et al., 2017). This heavy metal, compared to other metals, has been less explored in plant physiology (or physiological) studies. In addition, Co also influences N (symbiotic) absorption by being a structural part of leghemoglobin synthesis, which determines the activity of nodules in roots (WILSON; NICHOLAS, 1967).

In this way, our hypothesis is that there are benefits of the foliar application (spray) of Mo and Co under different availabilities (ionic strength, IS) from a single N (nitric presence $CaNO_3^{2-}$ / ammonium absence $NH_4H_2PO_4$) source of root application (hydroponic cultivation) on nutritional balance during the formation of seedling at initial phenological stage. The aims were to reveal if early growth phenological stages respond to Co/Mo spray under the condition of NO_3^- starvation and NH_4^+ deprivation and to evaluate the leaf application of mineral elements molybdenum (Mo) and cobalt (Co) in yellow passion fruit cultivated under different availabilities (ionic strength, IS) of Hoagland and Arnon No.01 nutrient solution mineral.

Material and methods

Plant material and experimental procedure

The present study was conducted in an experimental area belonging to the Federal University of São Carlos (*UFSCar*) Lagoa do Sino *campus*, municipality of Buri/SP, at height of 596 meters above sea level and with the following geographical coordinates: Latitude (23°47'57" S), and Longitude (48°35'15''). Ripe yellow passion fruits (*Passiflora edulis* Sims f. *flavicarpa* Degener) were collected from a single-mother plant, manually pulped with the aid of a sieve and running water and, after washing, seeds were kept in a drying bench. Seeds were submitted the phytosanitary treatment carried out in polystyrene trays filled a medium texture vermiculite substrate. Seedlings were transplanted to pots as soon as they presented the first pair of fully expanded leaves.

Treatments

Young plants were transplanted to plastic tubes with 0.3 L volumetric capacity and filled with medium texture vermiculite substrate. Previously, the Hoagland and Arnon No. 01 nutrient solution was improved (HOAGLAND; ARNON, 1950), being composed of unique nitrogen (nitrate) source ($CaNO_3^{2-}$) and absence of ammonium (NH_4^+) and used in the hydroponic technique with small methodological adjustment, according to Campos and collaborators (CAMPOS; VIEIRA; AMARO; DELACRUZ-CHACÓN et al., 2019). The different Hoagland's electrical conductivities (EC) employed were 25% (0.25 dSm^{-1}), 50% (1.00 dSm^{-1}) and 100% (2.00 dSm^{-1}) ionic strength (IS) (Table 1). Co and

Mo foliar application (Co/Mo spray) was prepared with cobalt(II) sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$, molecular weight 281.10 $g.mol^{-1}$) and sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$, molecular weight 205.92 $g.mol^{-1}$) in aqueous ion solutions of 0.3 $mL.L^{-1}$ (300 ppm) and 4.0 $mL.L^{-1}$ (4000 ppm), respectively. Aqueous ion solutions were applied in young plants with the aid of costal sprayer with CO_2 cylinder and pressure gauge measurement through a single fan tip (*MagnoJet*®, model AD/T 110 02), according to manufacturer's recommendations. The description of treatments is the following: T1 = 25%IS without Co/Mo spray; T2 = 50%IS without Co/Mo spray; T3 = 100%IS without Co/Mo spray; T4 = 25%IS plus Co/Mo spray; T5 = 50%IS plus Co/Mo spray; T6 = 100%IS plus Co/Mo spray.

Plant growth and growth analysis

Plant height, plant length, stem diameter, and leaf area were measured in 17 replicates, at 8 collection times (15, 30, 45, 60, 75, 90, 105, and 120 days after transplantation - DAT). In addition, 3 replicates (plants) from each treatment were used at 120 DAT to separate into shoots and roots. Subsequently, leaf, stem, and roots were dried in a forced convection benchtop oven (65 ± 2ºC), for 72h until constant dry matter measured with the aid of analytical scale with 0.001g sensitivity to obtain leaf dry matter (LDM), stem dry matter (SDM) and root dry matter (RDM) (BENINCASA, 1986).

Table 1. Hoagland and Arnon's nutrient solution n°1 (absence ammonium source, NH_4^+) containing macronutrients, with different nitrogen concentrations, micronutrients, and iron-EDTA solution.

Macronutrients	Stock solution (M)	25% N ($mL.L^{-1}$)	50% N ($mL.L^{-1}$)	100% N ($mL.L^{-1}$)
KH_2PO_4	136.08	1.00	1.00	1.00
KNO_3	101.10	1.25	2.50	5.00
$Ca(NO_3)_2$	236.16	1.25	2.50	5.00
$Mg(SO_4) \cdot 7H_2O$	246.50	2.00	2.00	2.00
(0.5M) K_2SO_4	087.13	3.77	2.50	0.00
(0.01M) $CaSO_4$	001.36	300.00	200.00	000.00
Micronutrients	Stock solution ($g.L^{-1}$)	1.0	1.0	1.0
H_3BO_3	2.86			
$MnCl_2 \cdot 4H_2O$	1.81			
$ZnSO_4 \cdot 7H_2O$	0.22			
$CuSO_4 \cdot 5H_2O$	0.08			
$H_2MoO_4 \cdot H_2O$	0.02			
Iron-EDTA Solution*	Stock solution ($g.L^{-1}$)	1.0	1.0	1.0
Bissodic EDTA ($C_{10}H_{14}N_2O_8Na_2.2H_2O$)	26.10			
$FeSO_4 \cdot 7H_2O$	24.90			

*Solution catalyzed by 265.00 mL NaOH (M).

Photosynthetic pigments

The contents of chlorophylls (*a* and *b*) and carotenoids were measured on 6 replicates per treatment according to methodology of Sims and Gamon (SIMS; GAMON, 2002). The concentration (µg of pigment per g of fresh matter) of chlorophylls *a* and *b* and carotenoids were calculated according to the following equations:

I. Chlorophyll *a* = 0.01373. (A663) - 0.000897. (A537) - 0.003046. (A647);

II. Chlorophyll *b* = 0.02405. (A647) - 0.004305. (A537) 0.005507. (A663);

III. Carotenoid = [A470 - (17.1. (Cl*a*-Cl*b*) - 9.479. (Anthocyanin)] / 119.26.

Statistical methods

Plants were randomly distributed into 17 blocks (replicates) with 6 treatments. Treatments were composed of a factorial scheme with 3 ionic strengths (25, 50, and 100%) combined with 2 levels of Co/Mo foliar spray (present/absent). Firstly, the data were analyzed through descriptive statistics using profile charts, histograms, and mean plots (with mean standard errors). Regarding plant growth measurements, the analysis of variance (ANOVA) was performed within each collection time. On the other hand, data from destructive sampling (at 120 DAT) were evaluated by of covariance analysis (ANCOVA). Initial height and length (at 15 DAT) were used as covariates in the growth analysis and pigment contents, while diameter and initial stem length were used as covariates to dry matter data. The ANOVA and ANCOVA validity was verified through residual charts and normality and variance homogeneity tests (Shapiro-Wilk's and Brown-Forsythe's, respectively). Logarithmic transformation was necessary to normalize and homogenize data variance in the case of pigment contents. Correlations between pigments were studied by Pearson's linear correlation coefficient and tested using student's *t* distribution. The Scott-Knott test was used to describe the pattern of influence of treatments on growth responses. In the case of significant effects on ANCOVA, adjusted means were compared by Tukey's test. All statistical analyses were conducted with *R* software and significance tests were performed by setting $\alpha = 0.05$.

Results and discussion

Plant growth and growth analysis

Plant height and length measures were affected by NO_3^- availability (i.e., ionic strength, IS) and Co and Mo foliar application (Co/Mo spray) at the majority of plant collection times (Table 2, $P<0.05$). However, there was no significant interaction between IS and spray application ($P>0.05$), with the exceptions of 60 DAT for plant height and 60 and 75 DAT for plant height and plant length. The effects of experimental factors on stem diameter seem to be occasional, and no consistent response pattern was observed over time (Table 2). Concerning leaf area, there was an effect of NO_3^- availabilities at almost all collection times (Table 2, $P<0.05$). However, no effect of foliar spray application and significant interactions between IS and spray were observed ($P>0.05$). The Scott-Knott test was used to group treatments with the same response pattern at each collection time (Figure 1). Considering plant height and length, treatment T6 (100%IS plus Co/Mo spray) provided the highest values at all collecting times, with the other treatments being combined in a group marked for lower results ($P<0.05$). In relation to stem diameter, all treatments were grouped until 90 DAT; but after that, treatment T6 presented the highest values ($P<0.05$), and the other treatments provided lower results. Considering leaf area, T6 showed better results from 75 DAT in comparison to the other treatments ($P<0.05$); however, T3 (100%IS without Co/Mo spray) showed intermediate plant growth, being grouped together with T6 from 90 DAT. Thus, plant growth responds to NO_3^- increases, even in the absence of NH_4^+, and the Co/Mo spray provided additional growth, ensuring better plant establishment and mitigating the effects of NH_4^+ deprivation. Except for SDM, no significant effects on growth analysis were found (ANCOVA, $P>0.05$). Significant interaction between Co/Mo spray and IS's was observed (ANCOVA; $F = 7.702$, degree-of-freedom = 2/10, $P=0.009$). In low EC (25%IS), SDM showed similar results regardless of Co/Mo spray (Figure 2). However, SDM showed higher value under Co/Mo spray when compared with control at 'moderate' EC (50%IS), although the mean difference was not statistically significant ($P>0.05$). In high EC (100%IS), the pattern was inversed, and mean SDM value under foliar Mo and Co applications was lower than that of control (Figure 2; $P<0.05$).

Photosynthetic pigments

Photosynthetic pigments (cl*a*, cl*b,* and car) were linear and positively correlated (Figure 3; $P<0.05$). Also, pigment data were asymmetrically distributed and, therefore, Box and Cox's transformations were used before inferential analyses. In low EC (25%IS), pigment content was considerably higher in the absence of Co/Mo spray (Figure 4) and significant interactions between EC and Co/Mo spray were observed ($P<0.05$; Table 3). In addition, significant main effect of Co/Mo spray on cl*a* and car was also observed, although no significant effects of EC on pigment content was observed. Plants treated with Co/Mo spray show mean cl*a* contents statistically equal at 25%IS, 50%IS, and 100%IS (Figure 4). Meanwhile, for cl*b* and car, no differences between 50%IS and 100%IS were found; however, cl*b* and car contents were statistically smaller at 25%IS when compared to 100%IS ($P<0.05$). This physiological pattern is the opposite when compared to ISs in the absence of Co/Mo spray, because pigment contents were higher at low EC (25% IS), while mean pigment contents were equal at 50%IS and 100% IS (Figure 4).

Our study verified the benefits of Co/Mo spray in the initial phenological growth stage of yellow passion fruits cultivated in Hoagland and Arnon nutrient No.1 solution (NH_4^+ deprivation). Nitrogen (N) plays an important role in plant growth and mediation of root and shoot development, which main forms are NO_3^- and NH_4^+, and between these two forms, NO_3^- is the most absorbed, as it is the most available mineral N form in the soil or nutrient solution (HAWKESFORD; HORST; KICHEY; LAMBERS et al., 2012). However, it remains unclear if crops can change their preference during their early phenological growth stages. The present study indicates that plant growth responses are severely affected by the absence of the NH_4^+ source. NO_3^- requires higher metabolic energy (use of ATP) for its assimilation in organic matter when comparing to NH_4^+. Since NO_3^- is absorbed in the oxidized form, and subsequently reduced to ammonia (NH_3) to be incorporated in the form of amino acids (glutamate). Unlike NH_4^+, NO_3^- is readily absorbed for later incorporation and, consequently, NH_4^+ demands lower metabolic expenditure. Usually, plants prefer NH_4^+ as the major nitrogen (N) source, because the uptake of ^{15}N from $^{15}NH_4^+$ is more quick than that from $^{15}NO_3^-$, as there is a more efficient expression pattern of NH_4^+ protein carrier compared with NO_3^-. In plants, NH_4^+ accumulated more than NO_3^- (TANG; LIU; ZHANG; MA et al., 2020).

Table 2. Analysis of variance results for plant growth as a function of time (days after transplanting), Co/Mo leaf application (spray), and N doses in young *Passiflora edulis* Sims. f. *flavicarpa* Degener plants (passion fruit).

Source of variation	D.F.⁺	**Days after transplanting**															
		15		30		45		60		75		90		105		120	
		F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
								Plant height (cm)									
N doses	2	1.89	0.1571	3.26*	0.0428	4.34*	0.0157	7.03*	0.0014	4.65*	0.0118	2.89	0.0606	5.25*	0.0068	5.52*	0.0054
Spray	1	9.55*	0.0026	7.76*	0.0064	4.93*	0.0288	7.08*	0.0091	4.01*	0.0479	2.80	0.0976	9.31*	0.0029	9.03*	0.0033
Interaction	2	0.58	0.5639	1.34	0.2660	1.51	0.2254	3.70*	0.0282	4.08*	0.0200	2.78	0.0674	1.89	0.1574	2.75	0.0688
								Plant length (cm)									
N doses	2	2.44	0.0927	3.11*	0.0490	2.65	0.0761	4.08*	0.0198	4.66*	0.0117	4.04*	0.0207	4.27*	0.0167	3.30*	0.0413
Spray	1	11.48*	0.0010	10.25*	0.0019	7.26*	0.0083	9.66*	0.0025	8.73*	0.0039	7.85*	0.0062	5.29*	0.0236	3.47	0.0655
Interaction	2	1.85	0.1627	2.42	0.0940	1.93	0.1510	2.01	0.1396	3.17*	0.0464	2.87	0.0616	1.92	0.1515	1.24	0.2931
								Stem diameter (mm)									
N doses	2	1.72	0.1841	0.68	0.5079	0.47	0.6245	0.71	0.4899	0.52	0.5953	1.36	0.2623	3.50*	0.0343	2.07	0.1321
Spray	1	3.87	0.0522	3.97*	0.0493	0.00	0.9873	0.09	0.7671	5.68*	0.0191	1.96	0.1650	0.61	0.4357	0.89	0.3467
Interaction	2	2.42	0.0943	1.02	0.3641	1.58	0.2104	0.42	0.6554	0.55	0.5802	1.44	0.2413	3.91*	0.0233	3.00	0.0545
								Leaf area (dm^2)									
N doses	2	3.27*	0.0424	0.41	0.6676	5.65*	0.0048	4.99	0.0087	3.77*	0.0267	6.41*	0.0024	9.75*	0.0001	11.05*	<0.0001
Spray	1	3.60	0.0607	1.93	0.1680	0.21	0.6452	0.62	0.4323	1.20	0.2752	0.13	0.7189	0.28	0.5980	0.33	0.5651
Interaction	2	2.42	0.0942	0.79	0.4549	1.29	0.2807	2.19	0.1172	1.50	0.2289	1.81	0.1685	0.87	0.4216	0.84	0.4311

⁺D.F. = Degree-of-freedom. ANOVA was performed with 96 degree-of-freedom to estimate for residuals variation. * Significant effects and interactions at α 0.05.

Table 3. Analysis of variance and covariance (ANCOVA) results for leaf pigment contents at 120 DAT in 6 replicates (n = 6) by treatment of *Passiflora edulis* Sims f. *flavicarpa* Degener seedlings (passion fruits) (data transformed by the logarithmic function).

Source of variation	D.F.⁺	Chlorophyll *a*		Chlorophyll *b*		Carotenoid	
		F	P	F	P	F	P
Initial height	1	9.37*	0.0048	12.00*	0.0017	11.53*	0.0021
Initial length	1	0.67	0.4201	1.23	0.2760	1.28	0.2667
Foliar spray	1	15.04*	0.0006	2.05	0.1632	9.52*	0.0045
N availabilities	2	2.36	0.1127	1.45	0.2527	0.36	0.6990
Spray x N availabilities	2	9.68*	0.0006	13.52*	0.0001	8.80*	0.0011

⁺D.F. = degrees-of-freedom. * Significant F values at level of α 0.05. Residual degrees of freedom = 28.

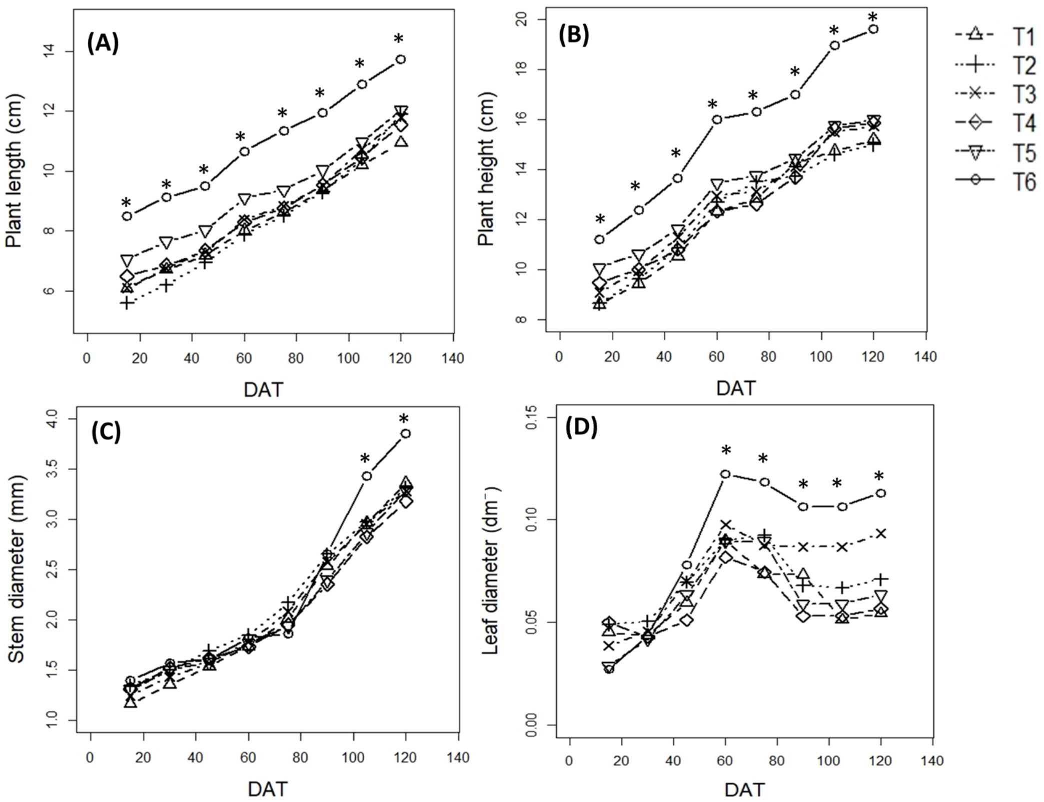

Figure 1. Growth analysis in young *Passiflora edulis* Sims. f. *flavicarpa* Degener plants (passion fruit) measures over time (days after transplanting - DAT) for different N ionic strength and foliar Mo and Co application conditions (T1, T2, and T3 are the 25, 50 and 100% IS without spray; and T4, T5, and T6 are 25, 50 and 100% IS with spray). Mean values from N=17 replicates within each time. Evaluations in which there was group formation of means by the Scott-Knott method (P<0.05) are identified by asterisks (*).

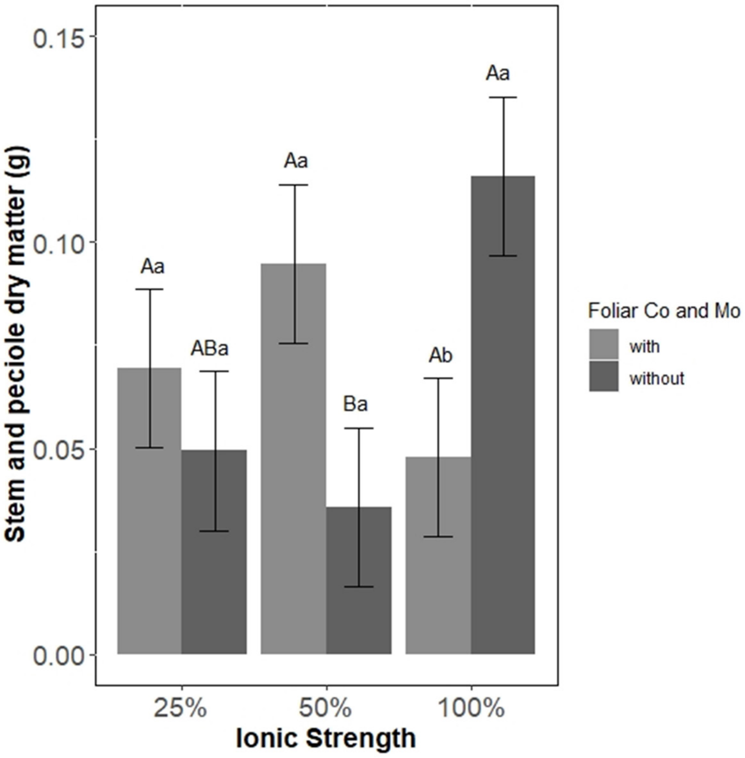

Figure 2. Stem and petiole dry matter of young *Passiflora edulis* Sims. f. *flavicarpa* Degener (passion fruit) plants as a function of foliar application and N ionic strength (Mean ± Standard Error). Distinct letters indicate means statistically different by the Tukey's test with α = 0.05. Uppercase letters are used to compare ionic strengths for different foliar application conditions, while lowercase letters compare foliar application levels within each ionic strength.

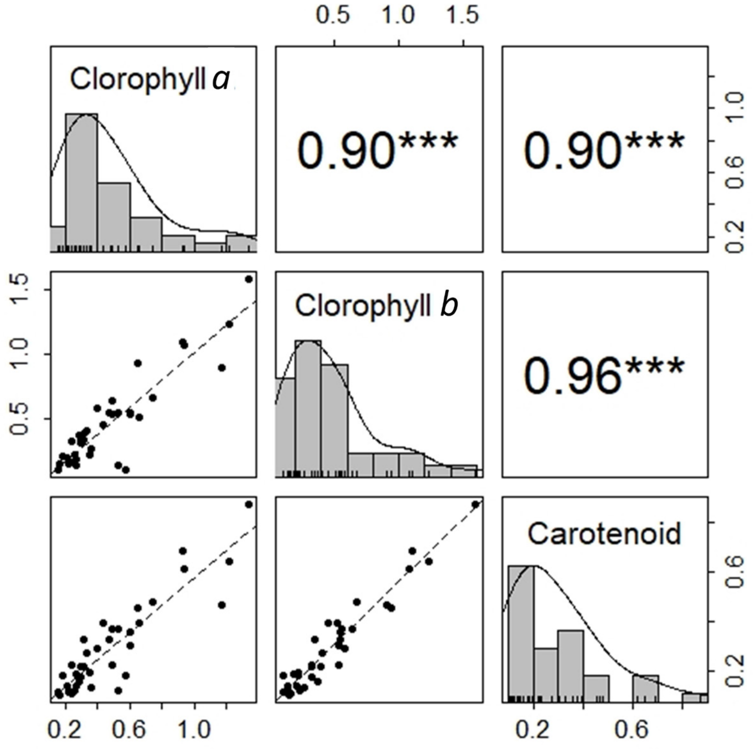

Figure 3. Distribution for chlorophyll *a*, chlorophyll *b*, and carotenoid pigment contents (mg/g) in *Passiflora edulis* Sims. f. *flavicarpa* Degener fruits (passion fruits). ***Pearson linear correlations were significant at level of alpha = 0.01

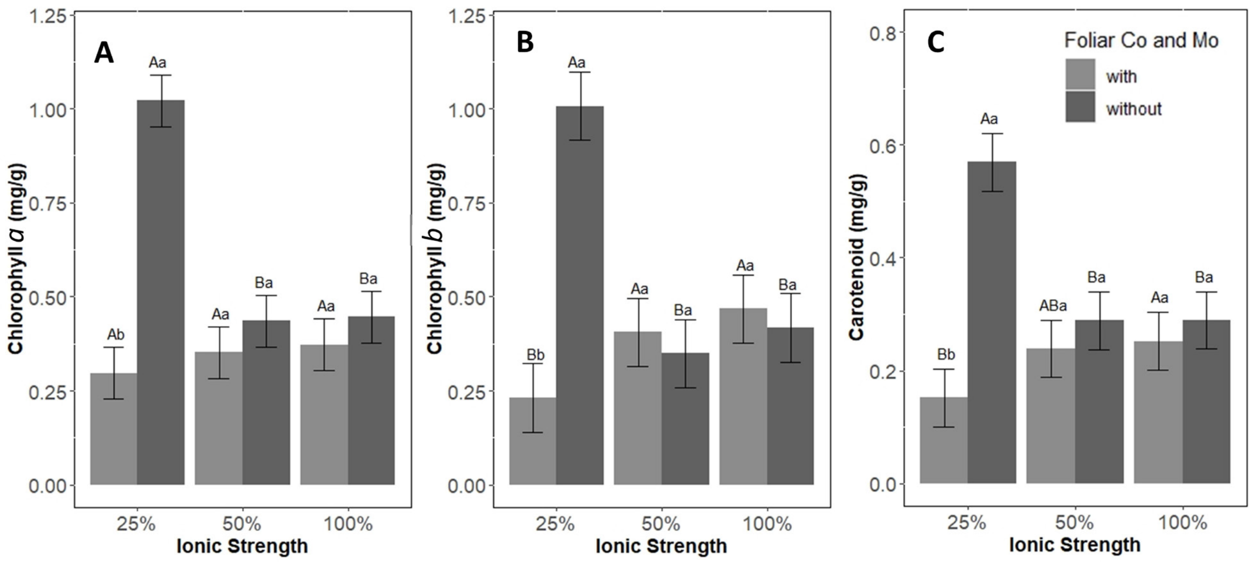

Figure 4. Contents of chlorophyll *a*, chlorophyll *b,* and carotenoid pigments of young *Passiflora edulis* Sims. f. *flavicarpa* Degener plants (passion fruits) for different N supply and Mo and Co foliar application conditions (Mean ± Standard Error, n=6 replicates). Distinct letters indicate means statistically different by Tukey's test with α = 0.05. Uppercase letters are used to compare ionic strengths for different foliar application conditions, while lowercase letters compare foliar application levels within each ionic strength.

Remarkably, higher NH_4^+ concentrations cause reduction in dry matter mass production, as the plant will not be able to promptly incorporate all N-NH_4^+ carbon skeleton (absence of carbon skeleton), and with alkaline pH, it would convert into NH_3, which would cause cell phytotoxicity. Higher NH_4^+ concentrations were reported in root tissue in comparison with NO_3^- treatment, whereas plants maintained a concentration equilibrated of NH_4^+ and NO_3^- in leaf tissue (RUAN; WEI; WANG; CHENG et al., 2016).

The use of the soilless cultivation technique is an excellent tool to assist in the manipulation of nutrient availability used in the roots of crops supplied via roots and to determine the nitrogen use efficiency (FAGERIA; BALIGAR, 2008). Nitrogen use efficiency (NUE) of eudicot angiosperms decreased at the early stage but increased later, on the other hand, for monocots, it decreased throughout the phenological growth stage (HAILE; NIGUSSIE; AYANA, 2012). Species that differed in N use shows different plant plasticity towards N source during the phenological growth stage. Legumes preferred NO_3^- throughout their growth while grasses preferred NH_4^+ at the early stages but later switched the preference for NO_3^- (CUI; YU; QIAO; XU et al., 2017) and higher plasticity may favor this species to mitigate N starvation stress. Inadequate N availability (i.e., unique N source) shows a morphophysiological change, assimilates partitioning and increases the root dry matter ratio (ZHU; CHEN; CHAN; YANG et al., 2018). However, in the present study, the experimental group treated with Co/Mo spray showed higher growth measurements cultivated in higher EC (100%IS), while Co/Mo spray showed no relevant effects under low EC (25%IS). Tomato (*Lycopersicon esculentum* L.) and tobacco (*Nicotiana tabacum* L.) are among the investigated species that have impaired vegetative development by adopting a single N source (RAHAYU; WALCH-LIU; NEUMANN; RÖMHELD *et al.*, 2005; WILLMANN; THOMFOHRDE; HAENSCH; NEHLS, 2014).

N absorption and assimilation by plants are multiregulated processes and integrated with the general plant metabolism. The multi-regulation of N metabolism impairs the identification of specific metabolic points that are more limiting for increasing productivity. In addition to differential nutrient uptake, the use of N may also vary as a function of the NO_3^- and NH_4^+ proportion in the root surface, as NO_3^- to be assimilated needs to be reduced in a process mediated by nitrate reductase enzymes (EC 1.6.6.1) and nitrite reductase (EC 1.7.7.1), while NH_4^+ does not require this step to be assimilated into amino acids (BOSCHIERO; MARIANO; AZEVEDO; OCHEUZE TRIVELIN, 2019; WANG; MIAO; LI, 2015). NH_4^+deprivation stress in tomato plants (*Solanum lycopersicum* L.) is responsible for rapid leaf growth inhibition associated with decline in cytokine concentration in xylem exudate and younger leaf tissue. According to Glanz-Idan and collaborators (GLANZ-IDAN; TARKOWSKI; TUREČKOVÁ; WOLF, 2020), NO_3^- is a direct player to stimulate cytokinin accumulation in shoots, with leaf growth regulation. Thus, N starvation or its low availability leads to leaf growth inhibition due to the decline of CK concentration (RAHAYU; WALCH-LIU; NEUMANN; RÖMHELD et al., 2005).

Regarding pigment contents, increased exposure of Co to leaves may cause damage to cell structural membranes (plasmalemma), such as inhibition of photosynthetic pigment synthesis. Co crosses the plant cuticle of air organs and after penetration is transported to other plant structures through the phloem in the same way as photosynthesis (SHAHID; DUMAT; KHALID; SCHRECK et al., 2017). The availability of Co in amounts from 100µM causes toxic effects such as reduced cell division, discoloration, and leaf abscission (LANGE; FAUCON; DELHAYE; HAMITI et al., 2017). In addition, leaf abscission in treatments deprived of ammonium (NH_4^+) source is a natural consequence of plant growth stress using a single (NO_3^-) nutritional N source (CAMPOS; VIEIRA; AMARO; DELACRUZ-CHACÓN et al., 2019). Certain abiotic stress conditions (environmental and/or nutritional), such as N starvation, may alter the phytohormonal balance and osmoregulation in the plant cell. Conversely, in NO_3^--fed plants, phenological growth stages may be delayed due to excessive concentrations of the plant cytokinin hormone (CK), since NO_3^- ion enhances active forms of this phytohormone (GARNICA; HOUDUSSE; ZAMARREÑO; GARCIA-MINA, 2010). With NH_4^+ deprivation in the root medium, plants develop a two-phase response.

In the first phase, the leaf elongation rate is reduced without affecting photosynthesis (ANANDACOOMARASWAMY; DE COSTA; TENNAKOON; VAN DER WERF, 2002). Root growth is maintained or even stimulated by the transport of assimilated carbon to roots, which results in lower shoot/root biomass ratio. Concomitantly, N compounds, particularly NO_3^-, are mobilized to maintain N metabolism and the capacity to absorb NO_3^- from the soil is increased. In the second phase, upon continued N starvation, the breakdown of leaf nucleic acids and proteins is triggered and, usually, associated with leaf senescence (HÖRTENSTEINER; FELLER, 2002).

However, the present study reported that plants treated with Co/Mo spray at 100%IS ensured the permanence of leaves compared to plants without Co/Mo treatment Co element play an important role in delaying leaf senescence by preventing chlorophyll degradation via blockage of the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC), by ACC oxidase, into plant hormone ethylene (Et) (DIVTE; YADAV; JAIN; PAUL et al., 2019). In addition, the understanding of this physiological inhibition mechanism is not yet elucidated, but it is known that Co forms a stable compound with the sulfhydryl group of this enzyme, blocking this action. According to Hänsch and Mendel (HÄNSCH; MENDEL, 2009), the aldehyde oxidase is a molybdo-flavoenzyme derived from the duplication of the xanthine dehydrogenase gene and both use the same prosthetic groups (FAD^+, Fe-S, and Mo-cofactor). This biochemical mechanism is crucial to obtain the biological function necessary to plant growth (MENDEL; KRUSE, 2012). In this way, we can speculate that the presence of Mo spray combined with nutrient solution composed of a single nitrogen (nitrate) source (absence of ammonium) promotes enzymatic catalysis of plant hormone auxin (AX) and benefits plant establishment during early phenological growth stages.

Conclusion

We concluded that the application of foliar Co and Mo spray combined with nutrient solution composed of a single nitrate source (absence of ammonium) did not affect dry matter accumulation, but the Co/Mo treatment alleviate NO_3^- starvation / NH_4^+ deprivation stress during the initial phenological growth stages of *Passiflora edulis* Sims. f. *flavicarpa* Degener.

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