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Scientific Article

EFFECT OF FREE AND NANOENCAPSULATED NITRIC OXIDE DONOR ON THE RESPONSE OF TREE PLANTS DURING THE HARDENING OFF PROCESS

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ABSTRACT

Nitric oxide (NO) plays a crucial role as a signaling molecule in several biological processes in plants, participating in the response to different types of environmental stresses. The technique of nanoencapsulation of NO donors appears as a promising approach to expand and enable the exogenous application of NO nitric oxide in environmental restoration programs. Therefore, this study aimed to verify the effect of using a NO donor, in free and encapsulated form, on the hardening off of seedlings of three tree species: *Hymenaea courbaril* L., *Amburana cearensis* (Allemão) A.C. Smith, and *Hymenaea stigonocarpa* Mart. ex Hayne. The seedlings were grown for 3 months under moderate shade and translocated to the hardening off sector, where they remained for 3 months under the treatments: chitosan nanoparticles containing S-nitrosoglutathione (NP-CS-GSNO) at concentrations of 0.025, 0.5, 0.1, and 0.2 mM, free S-nitrosoglutathione, at concentrations of 0.1 and 0.2 mM, or the Control. For *H. stigonocarpa*, treatments with free or nanoencapsulated GSNO did not differ from each other, differing only from the Control. *H. courbaril* presented similar behavior in relation to growth variables, however, in the analysis of physiological variables, only the NP-CS-GSNO 0.1 and 0.2 mM treatments differed from the other treatments. *A. cearensis* maintained similar behavior to *H. courbaril*, with only a difference in the Control treatment in relation to the other treatments. These results indicate that GSNO presents beneficial physiological effects when made available to native forest species, such as *H. stigonocarpa*, *H. courbaril*, and *A. cearensis*, triggering protective and incremental activities regarding photosynthesis, stomatal conductance, and biomass formation.

Keywords: Nanotechnology; Environmental stress; Acclimation

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EFEITO DO DOADOR DE ÓXIDO NÍTRICO LIVRE E NANOENCAPSULADO NA RESPOSTA DE PLANTAS ARBÓREAS EM SEU PROCESSO DE RUSTIFICAÇÃO

RESUMO – O óxido nítrico (NO) desempenha um papel crucial como molécula sinalizadora em diversos processos biológicos em plantas, participando da resposta a diferentes tipos de estresses ambientais. A técnica de nanoencapsulação de doadores de NO surge como uma abordagem promissora para expandir e viabilizar a aplicação exógena de óxido nítrico em programas de restauração ambiental. Assim, este estudo teve como objetivo verificar o efeito do uso de um doador de NO, nas formas livre e encapsulada, na aclimatação de mudas de três espécies arbóreas: *Hymenaea courbaril* L., *Amburana cearensis* (Allemão) A.C. Smith, e *Hymenaea stigonocarpa* Mart. ex Hayne. As mudas foram cultivadas por 3 meses sob sombra moderada e transferidas para o setor de rustificação, onde permaneceram por 3 meses sob os tratamentos: nanopartículas de quitosana contendo S-nitrosoglutationa (NP-CS-GSNO) nas concentrações de 0,025, 0,05, 0,1 e 0,2 mM; S-nitrosoglutationa livre, nas concentrações de 0,1 e 0,2 mM; e Controle. Para *H. stigonocarpa*, os tratamentos com GSNO livre ou nanoencapsulado não diferiram entre si, diferenciando-se apenas do Controle. *H. courbaril* apresentou comportamento semelhante em relação às variáveis de crescimento; entretanto, na análise das variáveis fisiológicas, apenas os tratamentos NP-CS-GSNO 0,1 e 0,2 mM diferiram dos demais tratamentos. *A. cearensis* manteve comportamento similar a *H. courbaril*, com diferença apenas no tratamento Controle em relação aos demais. Esses resultados indicam que o GSNO apresenta efeitos fisiológicos benéficos quando disponibilizado para espécies florestais nativas, como *H. stigonocarpa*, *H. courbaril* e *A. cearensis*, desencadeando atividades protetoras e incrementais em relação à fotossíntese, condutância estomática e formação de biomassa.

Palavras-Chave: Nanotecnologia; Estresse ambiental; Aclimatação

1. INTRODUCTION

In the contemporary context, the preservation of biodiversity and the mitigation of anthropogenic impacts on ecosystems are emerging as scientific and environmental imperatives. The degradation of ecologically sensitive areas, and increases in deforestation and climate change have heightened the urgency of taking energetic action to mitigate the damage caused and establish a sustainable ecological balance. Expansive livestock farming, intensive agriculture, and the excessive exploitation of natural resources have led to the deterioration of ecosystems that were once rich in biodiversity and ecosystem services. Uncontrolled deforestation is one of Brazil's main contributions to the effects of climate change.

In this scenario, the meticulous and numerous production of tree seedlings is consolidated as a structural strategy to reverse this worrying panorama. The careful introduction of tree seedlings in sites that are recognized as degraded is seen as a key step in the restoration of these areas. Trees not only contribute to soil stabilization and erosion control, but also promote the rehabilitation of endemic flora and fauna, creating microenvironments conducive to regeneration.

Studies focused on analyzing the response of tree seedlings to abiotic stress factors, as well as evaluating strategies used to improve their stress tolerance, are therefore essential to help increase the success of forest restoration and/or recovery.

Studies have demonstrated the role of nitric oxide (NO) as a signaling molecule in inducing plant tolerance to drought, since its small size, redox properties, and hydrophobic character allow its effective participation in the regulation of plant growth and development, as well as in responses to stress (Kolbert et al., 2021a).

However, NO donors are susceptible to environmental factors such as temperature, light, and pH, which lead to rapid and excessive release of NO and loss of its beneficial effects (Silveira et al., 2021). To this end, nanotechnology offers new agricultural applications that have focused on research into the activation of secondary metabolism in plants and the use of nanoparticles as a defense against harsh environmental conditions,

suitable for improving plant characteristics and their implementation in silvicultural practices (Becerra et al., 2022).

Thus, the objective of the current study was to evaluate the effect of treatment with chitosan nanoparticles containing S-nitrosoglutathione (NP-CS-GSNO) and free S-nitrosoglutathione (free GSNO) on the morphophysiological responses of jatobá-da-mata seedlings (*Hymenaea courbaril* L.), amburana (*Amburana cearensis* (Allemão) A.C.), and jatobá-do-cerrado seedlings (*Hymenaea stigonocarpa* Mart. Ex Hayne), compared with the control treatment in seedlings undergoing hardening in a forest nursery.

2. MATERIAL AND METHODS

2.1 Preparation of tree seedlings

The fruits of *H. courbaril* were harvested from mother trees measuring 10-12 m in height located in the municipality of Primeiro de Maio/PR (22º53'14''S, 50º56'42''W). The fruits of *H. stigonocarpa* and *A. cearensis* were collected in the Guanambi/BA region (14º13'01'' S, 42º46'40'' W).

All seedlings were produced in the forest nursery of the Laboratory of Biodiversity and Ecosystem Restoration – LABRE, belonging to the State University of Londrina – UEL, following the entire seedling production routine in the nursery, as recommended by Oliveira M et al. (2016). Sowing was performed indirectly, in boxes measuring 1,00 × 1,00 × 0,60 m, using sand as the only substrate. The seedlings were then transferred to 280 cm³ polypropylene tubes with commercial substrate (Ouro Negro®).

The seedlings remained in a forest nursery, under 50% shade with a shade cloth, with an automatic irrigation regime, three times a day for 30 minutes, until they reached the desired development conditions, an average of three months, with an average height of 15 cm for jatobá-da-mata and 10 cm for jatobá-do-cerrado and amburana. After this period, the plants were transferred to the hardening off section of the nursery, where they were kept in full sunlight for three months. The total time spent in the nursery was around 6 months, the time needed for the seedlings to reach a height of between 20 and 30 cm.

2.2 Preparation of nanocapsules (nps) and treatments

GSNO was synthesized and characterized according to the methodology of Silveira et al. (2016). Reduced glutathione (GSH) was dissolved in hydrochloric acid (1 mol L^{-1}) at 1.2 mol L^{-1}. An equimolar amount of sodium nitrite ($NaNO_2$) was added to the GSH solution in order to nitrosate GSH, in an ice bath for 30 minutes with magnetic stirring. Subsequently, acetone was added and this solution was filtered and washed several times with cold water, to obtain the precipitated GSNO. The solid obtained was lyophilized for 24 hours and stored at -20°C.

The NPs were prepared using the ionic gelation method (Marcato et al., 2013; Pelegrino et al., 2017). Briefly, chitosan (CS) was dissolved in acetic acid (1%) and 26 mmol L^{-1} of GSH were added to the solution. After 90 minutes of magnetic stirring at room temperature (25±2°C), a 0.6 mg mL^{-1} sodium tripolyphosphate (TPP) solution was added dropwise to the CS/GSH solution. The final mixture was magnetically stirred for at least 90 minutes, obtaining a final GSH concentration of 20 mmol L^{-1}.

To obtain chitosan NPs containing GSNO, an equimolar amount of sodium nitrite ($NaNO_2$) was added to the NP-CS/TPP-GSH suspension, followed by maintenance in the dark for 60 min. The final concentration of NP-CS/TPP-GSNO was 20 mM. The formulations were characterized by size, dynamic light scattering, NP tracking analysis, zeta potential, encapsulation efficiency, the polydispersity index (PDI), and pH.

All formulations were diluted in distilled water to obtain the desired concentrations. The plants were subjected to the concentrations 0.025, 0.05, 0.1, and 0.2 mM with NP-CS-GSNO; 0.1 and 0.2 mM in the GSNO-free donor condition; and the control treatment itself (0 mM).

Before the formulations were made available to the plants, all seedlings were raised to field capacity. They then received 30 mL of the formulation, applied directly to the plant substrate. This procedure was performed three times, on alternate days. The seedlings transferred to the environment under full sunlight only after the last supply of the solutions.

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2.3 Physiological parameters evaluated

Leaf gas exchange parameters were measured biweekly after the solutions were supplied. The youngest fully expanded leaf of each seedling was used for physiological analyses. Measurements were performed on sunny days between 7:00 and 10:00 a.m. Net photosynthesis (A) and stomatal conductance (gs) were determined based on the portable infrared gas analyzer model LICOR 6400 XT (Biosciences, Lincoln NE, USA), which was connected to a 6cm² chamber under saturated photosynthetically active radiation (1500 µmol $m^{-2}s^{-1}$).

Chlorophyll a fluorescence was measured in the middle third of the first fully expanded leaf, on the abaxial surface, avoiding the central vein, in the morning (between 7-10 am), with a 0S1p fluorometer (Opti Sciences), which allowed verification of the maximum efficiency of photosystem II (Fv/Fm ratio) and the existence of photoinhibition in intact leaves (Shimizu et al., 2006). The leaves were kept in the dark for 15 minutes with specific clips to measure the initial fluorescence (F0). The maximum fluorescence (Fm) was then analyzed after a saturating irradiance pulse, as well as the variable fluorescence (Fv) (Fv = Fm – F0).

Stomatal conductance measurements were performed in the middle third of the first fully expanded leaf, on the abaxial surface, in the morning (between 7-10 am), using an SC-1 porometer (METER Group).

2.4 Growth parameters

At the end of the experiment, morphological variables of all seedlings were analyzed. The stem diameter was measured when the plants were collected, using a digital caliper. Root height and length were measured using a millimeter ruler, and total leaf area (LA) was measured using a portable leaf area integrator, model LI-3000CAP (LiCor Inc., Lincoln, NE, USA). To determine dry mass, the different organs of the seedlings were dried in an oven at 60°C for 72h.

2.5 Statistical analysis

The design was completely randomized, with seven treatments (GSNO at dosages of 0.025, 0.5, 0.1, and 0.2 in nanoencapsulated format; 0.1 and 0.2 mM in free format; and the Control treatment) and six replicates. The data were subjected to analysis of variance and if significant, the means were analyzed using the Scott-Knott clustering test, at 5% significance ($p<0.05$). The assumptions of normality of errors and homogeneity of variances were tested by Shapiro-Wilk and Levene, respectively. All analyses were performed with the aid of R Software, using the AgroR (Shimizu et al., 2022).

3. RESULTS

After applying the different formulations, it was observed that for the species *H. stigonocarpa* the vegetative growth parameters did not differ from each other for the nanoencapsulated formulations and free GSNO, only the Control treatment presented lower values, differing from the other treatments (Table 1A).

The application of NP-CS-GSNO 0.2 mM promoted an increase in morphological parameters in relation to the Control, including leaf area, with an increase of 190.85%; shoot length (29.12%); stem diameter (31.18%); stem dry mass (44.04%); root dry mass (80.12%); leaf dry mass (163.15%); total dry mass (57.2%); and root size (36.05%).

For *H. courbaril* the behavior was similar, showing no difference between the different treatments supplying free or nanoencapsulated GSNO, and with the Control treatment generating the lowest values (Table 1B). For this species, the dosage of NP-CS-GSNO 0.1 mM stood out, leading to the greatest increase in the morphological variables analyzed in relation to the Control, with an increase of 80.35% in height, 78.39% in diameter, 20.37% in root length, 107.99% in leaf area, 129.14% in root dry mass, 129.69% in stem dry mass, 25% in leaf dry mass, and 94.77% in total dry mass.

The *A. cearensis* plants showed the smallest increases in the Control treatment. The other concentrations did not show a significant difference, however the 0.2 mM dosage stood out, leading to a considerable increase in relation to the Control treatment (Table 1C), with an increase of 54.99% in height, 49.05% in diameter, 32.5% in root size, 149.65% in leaf area, 102.92% in root dry mass, 128.67% in stem dry mass, 1035.43% in leaf dry mass,

and 165.17% in total dry mass.

Regarding the physiological parameters, a significant difference was observed between the treatments for stomatal conductance (gs) for *H. stigonocarpa*, as shown in Table 2A. According to Table 1, the Control treatment led to an increase in values in the initial conduction of the experiment, followed by a constant drop in the gs values, remaining among the lowest rates of all treatments. Contrary to the dosage of 0.2 mM NP-CS-GSNO, which initially presented unsatisfactory results in relation to the Control, followed by a rapid recovery, leading to the highest conductance values at the end of the experiment.

For treatments with free GSNO, only the 0.2 mM dosage showed satisfactory behavior, with a peak response in gs, favored by the rapid release of NO to the plants, followed by a subsequent drop and then increases in the results until the end of the analysis. In contrast, the 0.1 mM dosage, even though it also provided rapid NO availability, did not demonstrate any benefits for the plants in terms of gs values, possibly because the concentration did not produce a relevant effect. At the end of the experiment, the NP-CS-GSNO 0.2 mM treatment was 74.74% superior to the Control treatment.

The *H. courbaril* treatments differed from each other at the end of the experiment, with higher gs values for the treatments with nanoencapsulated GSNO when compared with the Control treatment and with the treatments with free GSNO (Table 2B). It can be observed that treatments with a dosage of 0.2 mM for NP-CS-GSNO and free GSNO maintained high gs values throughout the evaluation, an important fact for the initial development of the seedlings, since vigorous and resistant seedlings have greater chances of survival when transplanted in the field.

In comparative terms of the gs results of the NP-CS-GSNO 0.2 mM treatment with the Control treatment, there was a greater discrepancy between the observed values, since in the first evaluation, the plants which received the nanoencapsulated dosage of 0.2 mM presented a 61.6% higher value and in the last evaluation this increase rose to 173.12%, also differing from the treatment with 0.2 mM of free GSNO, with a 98.12% higher value.

A. cearensis plants showed greater prominence for gs with the NP-CS-GSNO 0.2 mM treatment (Table 2C), maintaining high values during the experiment, following a decline and approximation of values with the other treatments that also used nanoencapsulated GSNO in different doses. The gs value was 165.45% higher than the value attributed to the Control treatment.

An increase in A was observed for *H. stigonocarpa* in plants treated with free and nanoencapsulated GSNO in relation to the Control, showing a significant difference between treatments, with higher values recorded for NP-CS-GSNO 0.2 mM, which was 65.11% higher than the Control treatment. In contrast, *H. courbaril* did not show significant differences between treatments, with no increase in A observed in plants treated with NP-CS-GSNO and free GSNO in relation to the Control. There was no significant difference between treatments with NP-CS-GSNO and free GSNO for *A. cearensis*, however, these treatments differed from the Control treatment.

According to the chlorophyll a fluorescence analysis (Table 2 A, B, and C), the three species maintained a high Fv/Fm, close to 0.8 at dawn, although it can be seen that for the final reading the values did not differ statistically at the 5% probability level.

4. DISCUSSION

Providing nitric oxide (NO) to plants is a satisfactory practice, since nitric oxide plays a multifaceted role in plant physiology, influencing growth, development, stress response, and the ability to interact with the environment (Kolbert et al., 2021b). Its role as a signaling molecule is essential for the acclimation of plants to a wide range of environmental conditions and for optimizing their development.

As explained by Seabra et al. (2022), adequate delivery of NO to plant cells is still a challenge that hinders its use in natural field conditions, and since NO is a gaseous free radical with a short half-life under aerobic conditions, exogenous treatment with molecules that act as NO donors has been used as the main strategy to increase the endogenous NO content in plants and provoke the biological effects of NO. However, this strategy is often hampered by the relative instability of the donors, so a promising way to improve NO delivery to plants would be

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Table 1. Initial Development of *H. stigonocarpa* (A), *H. courbaril* (B), and *A. cearensis* (C) under full sunlight and with the addition of different formulations of GSNO, in both free and nanoencapsulated forms. Where: Alt. PA: shoot height, Diam: stem diameter, C.raiz: root length; AF: leaf area, MSr: root dry mass, MSc: stem dry mass, MSf: leaf dry mass, MStot: total dry mass, * Free S-nitrosoglutathione (GSNO)

Tabela 1. Desenvolvimento inicial de *H. stigonocarpa* (A), *H. courbaril* (B) e *A. cearensis* (C) sob sol pleno e com adição de formulações distintas de GSNO, na forma livre e nanoencapsulada. Onde: Alt. PA: altura de parte aérea, Diam: diâmetro do caule, C.raiz: Comprimento de raiz; AF: área foliar, MSr: massa seca de raiz, MSc: massa seca de caule, MSf: massa seca foliar, MStot: massa seca total, * S-nitrosoglutationa (GSNO) livre

A	Alt. PA cm	Diam mm	C. raiz cm	AF t cm²	MSr t g	MSc t	MSf t	MStot t
0,025	20,033 a	4,792 a	15,25 a	63,627 a	4,982 a	1,676 a	0,685 a	7,344 a
0,05	22,05 a	5,49 a	15,75 a	91,467 a	5,994 a	1,788 a	1,007 a	8,79 a
0,1	19,386 a	5,413 a	16,857 a	62,184 a	5,082 a	1,524 a	0,759 a	6,422 a
0,2	22,42 a	5,546 a	16,9 a	95,068 a	6,653 a	1,847 a	1,035 a	9,535 a
0,1*	21,1 a	5,462 a	15,6 a	72,668 a	5,146 a	2,238 a	0,933 a	8,317 a
0,2*	19,417 a	4,915 a	13,75 a	73,04 a	5,092 a	1,42 a	0,857 a	7,226 a
0	17,367 b	4,193 b	12,417 b	32,685 b	3,693 b	1,282 b	0,393 b	6,068 b
CV(%)	17,915	15,158	13,767	26,875	17,035	18,276	24,286	17,372
B	**Alt. PA cm**	**Diam mm**	**C. raiz cm**	**AF t cm²**	**MSr t g**	**MSc t**	**MSf t**	**MStot t**
0,025	32,7 a	6,152 a	15,833 a	240,068 a	3,652 a	3,359 a	2,188 a	9,2 a
0,05	28,457 a	6,34 a	15,286 a	277,059 a	2,635 a	2,908 a	1,371 b	6,914 b
0,1	36,317 a	7,423 a	16,25 a	295,789 a	3,967 a	4,642 a	2,331 a	10,94 a
0,2	32,05 a	6,993 a	14,5 a	287,567 a	3,107 a	3,59 a	2,269 a	9,967 a
0,1*	32,514 a	6,213 a	15,929 a	270,013 a	3,367 a	3,618 a	2,208 a	9,192 a
0,2*	32,243 a	5,983 a	15,5 a	209,619 a	2,133 a	2,749 a	1,179 b	6,439 b
0	20,14 b	4,158 b	13,5 a	142,19 b	1,731 b	2,021 b	1,865 b	5,617 b
CV(%)	29,293	19,952	9,132	33,551	25,414	25,912	30,785	25,173
C	**Alt. PA cm**	**Diam mm**	**C. raiz cm**	**AF t cm²**	**MSr t g**	**MSc t**	**MSf t**	**MStot t**
0,025	15,417 a	3,323 a	15,583 a	49,547 a	6,321 a	0,569 a	1,038 a	7,928 a
0.05	17,233 a	3,947 a	14,333 a	41,24 a	5,039 a	0,787 a	0,201 a	6,027 a
0.1	13,233 b	3,022 b	13,417 b	48,06 a	5,769 a	0,484 b	0,225 a	6,478 a
0.2	18,65 a	4,562 a	16,167 a	78,025 a	6,949 a	0,965 a	1,44 a	10,795 a
0,1*	16,78 a	3,908 a	15,6 a	69,512 a	6,772 a	0,697 a	0,271 a	7,74 a
0,2*	18,16 a	3,99 a	14,7 a	71,576 a	8,524 a	0,924 a	0,326 a	9,774 a
0	12,04 b	3,06 b	12,2 b	31,274 b	3,42 b	0,422 b	0,127 a	4,07 b
CV(%)	25,098	20,846	10,456	24,921	23,988	22,896	64,662	23,655

through NO-releasing nanomaterials (such as chitosan), to maintain a sustained NO delivery in the early development of plants.

In the current study, for the three forest species analyzed, no statistical difference was found between the treatments with nanoencapsulated and free GSNO in the analysis of A, with treated seedlings differing only from the Control treatment. A similar fact was also reported by Carmo et al. (2021), who, when analyzing Heliocarpus

Table 2. Physiological evaluations of *H. stigonocarpa* (A), *H. courbaril* (B), and *A. cearensis* (C) under full sunlight and with the addition of different formulations of GSNO, in both free and nanoencapsulated forms. Where: A: net photosynthesis, Fv/Fm: chlorophyll a fluorescence, gs: stomatal conductance, * Free S-nitrosoglutathione (GSNO). Temporal identification: A1-11/10/22, A2-24/10/22, A3-17/12/22, A4-13/01/22, Fv/Fm1-24/10/22, Fv/Fm2-31/10/22, Fv/Fm3-17/12/22, gs1-11/10/22, gs2-24/10/22, gs3-04/11/22, gs4-15/11/22, gs5-24/11/22

Tabela 2. Avaliações fisiológicas de *H. stigonocarpa* (A), *H. courbaril* (B) e *A. cearensis* (C) sob sol pleno e com adição de formulações distintas de GSNO, na forma livre e nanoencapsulada. Onde: A: fotossíntese líquida, Fv/Fm: fluorescência da clorofila a, gs: condutância estomática, * S-nitrosoglutationa (GSNO) livre. Identificação temporal: A1-11/10/22, A2-24/10/22, A3-17/12/22, A4-13/01/22, Fv/Fm1-24/10/22, Fv/Fm2-31/10/22, Fv/Fm3-17/12/22, gs1-11/10/22, gs2-24/10/22, gs3-04/11/22, gs4-15/11/22, gs5-24/11/22

A	A1t	A2t	A3 t	A4t	Fv/Fm1	Fv/Fm2
			µmol m^{-2} s^{-1}			
0,025	6,60 a	5,342 a	10,07 a	11,83 a	0,698 a	0,639 a
0.05	6,68 a	3,74 b	9,113 a	10,59 a	0,674 a	0,669 a
0.1	5,47 a	3,56 b	6,78 b	8,066 b	0,637 a	0,609 a
0.2	5,62 a	3,53 b	6,063 b	12,65 a	0,684 a	0,629 a
0,1*	5,876 a	3,957 b	7,012 b	9,75 a	0,691 a	0,619 a
0,2*	7,901 a	5,662 a	10,51 a	10,84 a	0,725 a	0,669 a
0	6,502 a	3,295 b	7,666 b	7,684 b	0,682 a	0,6 a
CV (%)	11,139	15,604	13,782	9,82	10,273	11,168
A	**Fv/Fm3**	**gs 1**	**gs 2**	**gs 3**	**gs 4**	**gs 5**
	µmol m^{-2} s^{-1}			**mol m^{-2} s^{-1}**		
0,025	0,786 a	147,3 b	130,98 a	187,583 a	220,6 a	157,75 a
0.05	0,791 a	137,53 b	118,88 a	144,18 b	164,4 a	148,73 a
0.1	0,739 a	114,22 b	84,58 b	116,08 b	123,71 b	129,88 b
0.2	0,768 a	135,12 b	127 a	135,24 b	144,8 b	193,92 a
0,1*	0,761 a	212,4 a	94,6 b	92,06 b	85 b	112,48 b
0,2*	0,784 a	272,667 a	114 a	126,35 b	164,167 a	171,033 a
0	0,776 a	169,33 b	90,967 b	100,31 b	105,6 b	111,033 b
CV (%)	4,111	41,735	31,649	37,806	37,856	34,31
B	**A1t**	**A2t**	**A3 t**	**A4t**	**Fv/Fm1**	**Fv/Fm2**
			µmol m^{-2} s^{-1}			
0,025	7,225 a	4,627 a	5,148 a	6,033 a	0,758 a	0,712 b
0,05	7,526 a	5,029 a	4,897 a	4,828 b	0,756 a	0,723 b
0,1	6,99 a	5,365 a	7,554 a	8,048 a	0,726 a	0,716 b
0,2	7,283 a	4,56 a	5,144 a	7,833 a	0,745 a	0,708 b
0,1*	7,958 a	5,31 a	4,814 a	6,545 a	0,765 a	0,757 a
0,2*	7,798 a	6,916 a	6,23 a	6,155 a	0,761 a	0,762 a
0	8,14 a	6,192 a	4,361 a	3,005 b	0,768 a	0,693 b
CV(%)	10,71	15,598	20,424	18,56	5,111	4,702

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B	Fv/Fm3	gs 1	gs 2	gs 3	gs 4	gs 5
	µmol m^{-2} s^{-1}			mol m^{-2} s^{-1}		
0,025	0,802 a	180,9 b	135,35 a	101,133 b	134,283 a	62,2 b
0,05	0,799 a	138,314 b	121,786 b	128,186 b	117,214 a	86,829 b
0,1	0,784 a	164,786 b	147,157 a	112,071 b	126,667 a	142,05 a
0,2	0,816 a	362,28 a	288,44 a	186,56 a	146,74 a	154,32 a
0,1*	0,819 a	194,586 b	160,829 a	136,957 b	84,071 b	75,686 b
0,2*	0,812 a	178,129 b	168,543 a	272,097 a	81,143 b	77,9 b
0	0,806 a	224,2 b	127,157 b	110,757 b	83,275 b	56,486 b
CV(%)	3,559	29,196	31,605	44,061	34,587	25,382
C	**A1t**	**A2t**	**A3 t**	**A4t**	**Fv/Fm1**	**Fv/Fm2**
	µmol m^{-2} s^{-1}					
0,025	4,862 a	2,842 a	5,094 a	4,594 a	0,751 a	0,752 a
0,05	4,464 a	2,248 a	4,134 a	4,002 a	0,742 a	0,73 a
0,1	4,486 a	2,612 a	5,932 a	5,843 a	0,736 a	0,718 a
0,2	4,527 a	3,13 a	5,395 a	6,292 a	0,74 a	0,711 a
0,1*	5,393 a	2,864 a	5,31 a	4,858 a	0,748 a	0,727 a
0,2*	5,269 a	3,148 a	5,122 a	4,369 a	0,716 a	0,69 a
0	4,732 a	2,112 a	2,2 a	2,326 b	0,706 a	0,714 a
CV(%)	8,464	15,888	14,639	18,487	5,327	6,2
C	**Fv/Fm3**	**gs 1**	**gs 2**	**gs 3**	**gs 4**	**gs 5**
	µmol m^{-2} s^{-1}			mol m^{-2} s^{-1}		
0,025	0,763 a	123,817 a	103,483 a	102,633 a	106,433 a	130,14 b
0,05	0,794 a	112,65 a	80,05 a	72,183 b	56,783 b	115,55 b
0,1	0,792 a	76,633 a	104,283 a	98,483 b	62,45 b	127,017 b
0,2	0,786 a	133,917 a	128,967 a	165,2 a	154,383 a	159,383 a
0,1*	0,8 a	144,54 a	99,94 a	63,86 b	60,4 b	66 c
0,2*	0,779 a	127,925 a	121,725 a	66,44 b	73,22 b	65,44 c
0	0,782 a	158,44 a	65,54 a	62,14 b	88,34 b	63,625 c
CV(%)	2,819	34,759	16,165	43,421	36,254	25,176

popayanensis plants under water stress, found that both free and nanoencapsulated S-nitroso-MSA prevented the deterioration of net photosynthesis, and highlighted the important contribution of NO to tree seedlings subjected to stress.

Although no statistical difference was observed between treatments with nanoencapsulated and free GSNO, a highlight was noted for NP-CS-GSNO 0.2 mM in A for the three forest species tested, as this concentration promoted recovery and elevation of A values in the plants. The 0.2 mM NP-CS-GSNO dosage behaved as reported by Lopes-Oliveira et al. (2019), in which encapsulation of the NO donor using nanomaterials (NP-CS-GSNO) was used as a strategy to protect these molecules from decomposition, allowing the controlled release of NO and prolonging its period of action (Table 2).

As the effects did not differ, both free and nanoencapsulated GSNO prevented A deterioration in plants under hardening off (Table 2). However, as pointed out by

Oliveira H et al. (2016), the effect of chitosan nanoparticles carrying the S-nitroso-MSA donor on corn plants subjected to saline stress was more efficient than the free donor, promoting a slower and more controlled release of NO, resulting in protection against the effects of high salinity. It is worth noting that in the cited work, the plants were grown in sand, under controlled conditions in a greenhouse, while the current study was carried out under cultivation conditions in a seedling nursery.

According to Lopes-Oliveira et al. (2021), throughout their life cycle, plants can face seasonal and sporadic deviations from ideal light conditions, including excessive or insufficient light intensity; both irradiances below the light compensation point and well above the light saturation point of photosynthesis, can lead to oxidative stress, photoinhibition, and limited plant growth and development, and these high levels of radiation, especially UV-B, can cause DNA damage, pigment photooxidation, inhibition of photosynthetic activity, and reduced biomass accumulation.

This light stress can increase the production of ROS, lead to lipid peroxidation, and cause damage to cell membranes, consequently inhibiting photosynthesis, respiration, and plant growth. In addition, high amounts of visible light or UV-B modulate the production of NO in plant cells, which in turn activates the antioxidant defenses of plants in these circumstances, promoting protection against these effects of light stress (Zhao et al., 2020; Lau et al., 2021). As observed, the supply of exogenous NO at all concentrations had a beneficial effect on the growth and photosynthetic activity of plants under stress.

In addition to A, other physiological events may benefit from the exogenous supply of NO, such as substomatal carbon dioxide concentration, transpiration rate, and stomatal conductance (Lau et al., 2021). Referring to stomatal conductance, it is clear that different results were observed for the positive effects of GSNO nanoencapsulation and the adoption of its free form, indicating that the physiological response is also dependent on the plant species (Lopes-Oliveira et al., 2019).

Mariyam & Seth (2023) argue that NO supplementation has shown surprising behavior in prominently influencing the photosynthetic and stomatal regulation of plants and, according to the authors, NO has gained a new place in plant science, aimed at stress physiology, due to its direct involvement in biotic and abiotic stresses.

Nitric oxide controls the process of stomatal opening and closing. This occurs because this compound interacts with metabolic signaling, including Ca^{2+}, which is highly active in the homeostatic control of cells, including guard cells. In addition, it has been proven that the association of NO with abscisic acid (ABA) can affect the amplitude of stomatal opening and closing (Ferraz, 2021). In some situations, NO can antagonize the effects of ABA, which tends to close stomata in plants under high irradiance (Lopes-Oliveira et al., 2021). This results in the opening of stomata and, therefore, an increase in stomatal conductance, favoring the increase in photosynthesis.

Regarding the growth variables, it is clear that the treatments with NO, free or nanoencapsulated, regardless of the concentration, promoted positive effects on the morphological parameters evaluated in relation to the control for the three forest species.

H. stigonocarpa showed no difference between treatments involving NO, with an increase in values for the dosage of 0.2 mM. *H. courbaril* showed no difference for the variables root size, size of the aerial part, diameter, leaf area, and dry masses of root and stem, while only the Control treatment differed from the others and the treatments with the dosages of NP-CS-GSNO 0.05 and 0.1 mM free GSNO did not differ from the control for the variables leaf dry mass and total dry mass. *A. cearensis* presented similar behavior to *H. stigonocarpa*, with only the NP-CS-GSNO 0.1 mM treatment not differing from the Control treatment in relation to the others.

An important point to be highlighted is that the current study was developed in a forest nursery, with controlled irrigation conditions, free from weed competition and attacks from ants and other agricultural pests, that is, an environment controlled to the mentioned stresses. This fact that can favor plant development in all treatments. It is also worth remembering that the supply of solutions with free and nanoencapsulated NO donor occurred approximately 24 h, prior to the plant's hardening off period, and after this period the plants were relocated to the environment under full sunlight.

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5. CONCLUSION

- GSNO, a NO donor, used in free and nanoencapsulated form, presents beneficial physiological effects when made available to the native forest species analyzed, triggering protective activities and increasing photosynthesis and stomatal conductance. However, we recommend a dosage of 0.2 mM of NP-CS-GSNO.

- The treatments with NP-CS-GSNO and free GSNO promoted the initial development of seedlings of the three native tree species. In general, these treatments did not differ from each other, demonstrating a difference only when compared to the results obtained with the control treatment. It is necessary to extend the analysis time of the initial development in the nursery to add greater veracity to the objectives set.

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AUTHOR CONTRIBUTIONS

Guariz, H. R.: Writing, conducting experiments, investigation, project administration; Oliveira, H. C.: Supervision, visualization, investigation, validation, review, editing; Shimizu, G. D.: Formal analysis, software development, validation; Pieretti, J. C.: Methodology, validation, review; Seabra, A. B.: Methodology, validation, review.

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