Revista de Ciências Agroveterinárias
Universidade do Estado de Santa Catarina (UDESC)

Research Article

Effect of nitrogen doses on morphological parameters and content of cumarina in accesses of *Mikania Glomerata* Sprengel (guaco)

Efeito de doses de nitrogênio sobre parâmetros morfológicos e conteúdo de cumarina em acessos de Mikania Glomerata Sprengel (guaco)

Luciano Alves*[1(ORCID 0000-0003-0565-5738)] **& Cícero Deschamps**[2(ORCID 0000-0003-0786-0532)]

[1]Instituto Federal Catarinense, Araquari, SC, Brazil. *Author for correspondence: luciano.alves@ifc.edu.br
[2]Universidade Federal do Paraná, Curitiba, PR, Brazil.

Submission: August 21th, 2024 | Acceptance: April 19th, 2025

ABSTRACT

Nitrogen plays a role in the synthesis of secondary metabolites. Guaco (*Mikania glomerata* Sprengel is a medicinal species used in the treatment of respiratory diseases due to its content of coumarin (1,2-benzopyrone). The aim of this study was to evaluate the effect of ammonium nitrate doses on morphological parameters and coumarin content in two guaco accessions. The experiment was conducted in a randomized block design, with a 2 x 5 factorial arrangement, where the treatments were the combination of two guaco accessions and five fertilization doses, including a control. The fertilization levels were 0 g pl^1; 35 g pl^{-1}; 70 g pl^{-1}; 140 g pl^{-1}; and 280 g pl^{-1}. At planting, 35 g pl^{-1} was applied as a top-dressing, with the remaining doses split into three applications at 30, 60, and 90 days after planting. The experiment was conducted for 18 months, with two analyses performed: one at 6 months after planting and another with plants that remained in the field for 18 months. After these periods, the following parameters were evaluated: dry mass of leaves, branches, and total; leaf area; and coumarin content. For all parameters analyzed and at both harvest times, a quadratic effect was observed in response to nitrogen doses for all variables studied. Nitrogen doses influenced the coumarin content in the evaluated accessions. The doses of maximum technical efficiency varied among the evaluated parameters, harvest times, and accessions. In the six-month cultivation, the guaco accessions showed higher coumarin values in kg ha^{-1}, as well as within the standards required by ANVISA, when cultivated with doses of 130 g N pl^{-1} for accession G1 and 106 g N pl^{-1} for accession G2.

KEYWORDS: *Mikania glomerata* Sprengel. Secondary metabolism. Growth analysis. Shrimp shell. 1,2-benzopyrone.

RESUMO

O nitrogênio desempenha um papel fundamental na síntese de metabólitos secundários. Guaco (*Mikania glomerata* Sprengel) é uma espécie medicinal utilizada no tratamento de doenças respiratórias devido ao seu conteúdo de cumarina (1,2-benzopirano). O objetivo deste estudo foi o de avaliar o efeito de doses de nitrato de amônio em parâmetros morfológicos e no teor de cumarina em duas amostras de guaco. O experimento foi conduzido em delineamento de blocos casualizados, com arranjo fatorial 2 x 5, onde os tratamentos foram a combinação de duas amostras de guaco e cinco doses de fertilização, incluindo o controle. Os níveis de fertilização foram 0 g pl^{-1}; 35 g pl^{-1}; 70 g pl^{-1}; 140 g pl^{-1}; e 280 g pl^{-1}. No plantio, foi aplicada uma dose de 35 g pl^{-1} como cobertura, com as doses restantes divididas em três aplicações aos 30, 60 e 90 dias após o plantio. O experimento foi conduzido por 18 meses, com duas análises realizadas: uma aos 6 meses após o plantio e outra com plantas que permaneceram no campo por 18 meses. Após esses períodos, foram avaliados os seguintes parâmetros: massa seca de folhas, galhos e total; área foliar; e teor de cumarina. Para todos os parâmetros analisados e em ambos os momentos de colheita, foi observado efeito quadrático em resposta às doses de nitrogênio para todas as variáveis estudadas. As doses de nitrogênio influenciaram o teor de cumarina nas amostras avaliadas. As doses de máxima eficiência técnica variaram entre os parâmetros avaliados, os momentos de colheita e as amostras. No cultivo de seis meses, as amostras de guaco apresentaram valores mais altos de cumarina em kg ha^{-1}, bem

Publisher's Note: UDESC stays neutral concerning jurisdictional claims in published maps and institutional affiliations.

This work is licensed under a Creative Commons Attribution 4.0 International License.

como dentro dos padrões exigidos pela ANVISA, quando fertilizadas com doses de 130 g N pl^{-1} para a amostra G1 e 106 g N pl-1 para a amostra G2.

PALAVRAS-CHAVE: *Mikania glomerata* Sprengel. Metabolismo secundário. Análise de crescimento. Casca de camarão. 1,2-benzopirona.

INTRODUCTION

In Brazil, there are few studies related to the cultivation of medicinal species addressing fertility aspects, as most of the material sold, particularly in the case of native species, is obtained through extraction (RODRIGUES et al. 2016).

In terms of mineral nutrition, nitrogen is one of the most important nutrients for plant metabolism because it is involved in the composition of a series of molecules, such as amino acids, proteins, enzymes, coenzymes, and chlorophyll. Limited availability or deficiency of this element can lead to reduced growth and yield losses (GIORGI et al. 2009, DE MOURA GUERRA et al. 2020).

Medicinal species, when subjected to limiting fertility conditions, exhibit a wide variation in the levels of active principles in the accumulating organs. For instance, in the case of alkaloids, nutrient suppression in Indian tobacco plants (*Lobelia inflata* Lineu) results in decreased levels of the active principle, whereas in poppy (Papaver somniferum), the behavior is reversed (DE MORAIS 2009).

In the case of phenolic compounds, and especially coumarins, there is a correlation between nitrogen availability in the soil and the levels of active principles in the plant. Generally, high levels of the active principle are found in plants grown under conditions of excess nitrogen (GOBBO-NETO & LOPES 2007).

This variation in active principle levels in response to nitrogen availability may be related to the increased activity of the enzyme phenylalanine ammonia-lyase (PAL), which is key in the synthesis of the metabolite (JONES & HARTLEY 1999).

According to GOBBO-NETO & LOPES (2007), it is important to emphasize that studies linking the interaction of nutritional factors with the production of secondary metabolites by plants are quite scarce and limited to exotic species, many of which originate from temperate regions and have economic importance. This does not reflect the behavior of wild species or those from other types of habitats, such as Guaco. Thus, there is a need for studies that help achieve uniform and high-quality raw materials.

Coumarins are a class of phenolic compounds classified as lactones of o-hydroxycinnamic acid, widely distributed among plant species, such as guaco (*M. glomerata* Sprengel.) (TEÓFILO & UHLMANN 2021).

With approximately 400 species distributed across Central and South America, the genus *Mikania*, which includes *M. glomerata* Sprengel., belongs to the Asteraceae family and is commonly known as "guaco," "cipó-caatinga," "guaco-cheiroso," and "erva-de-serpente." It is widely distributed in Brazil and its use is authorized by the Unified Health System (SUS), with the species included in the Brazilian Pharmacopoeia (TEÓFILO & UHLMANN 2021, UENO & SAWAYA 2017).

Among its various uses, guaco is popularly recommended for the treatment of bronchitis, asthma, and cough, primarily due to its bronchodilator effect, which is related to the presence of coumarin (1,2-benzopyrone) (DA COSTA SOUZA et al.

2022, TEÓFILO & UHLMANN 2021, SOUSA et al. 2023).

Additionally, the literature indicates the use of the species as an anti-inflammatory agent (FALCÃO et al. 2005), as well as due to its antimicrobial properties (OLIVEIRA & SATURNINO 2022).

The objective of this study was to evaluate the effect of nitrogen doses on growth parameters and coumarin content in two accessions of *M. glomerata* Sprengel. cultivated for periods of six and eighteen months in the municipality of Araquari, located in the northern region of the State of Santa Catarina.

MATERIALS AND METHODS

Two accessions of Guaco (*M. glomerata* Sprengel.), designated G1 and G2, were used. G1 originated from the Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI), while G2 came from the experimental farm of the Instituto Federal Catarinense - Araquari Campus.

The herbarium specimens were deposited at the Gerdt Hatschbach Botanical Museum Herbarium of the Municipal Government of Curitiba, under the numbers 367612 and 340947, corresponding to accessions G1 and G2, respectively.

The cuttings, each containing a node, were prepared with a bevel cut at the base and a straight cut above the last apical bud, resulting in an approximate length of 10 cm. Two leaves were left on the apical portion, with their area reduced by half. For disinf.ection, the cuttings were immersed in a 0.5% sodium hypochlorite solution for five minutes, followed by a two-minute rinse in running water. The bases of the cuttings were treated with indole butyric acid (IBA) at a concentration of 3000 mg l^{-1} in a 50% alcoholic solution for fifteen seconds. Planting was carried out in seedling production bags measuring 10 x 18 cm, filled with the commercial Carolina® substrate, which consists of sphagnum peat, expanded vermiculite, dolomitic limestone, agricultural gypsum, and traces of NPK fertilizer. The cuttings were kept for 45 days in a mist chamber (80% RH). Acclimatization was performed under 40% shade for 10 days, followed by full sun exposure for an equal period before planting.

The planting was carried out at the experimental farm of the Instituto Federal Catarinense, Araquari Campus, in a soil classified as quartzarenic neosol. The soil characteristics in the arable layer (0-20 cm) were as follows: pH CaCl2: 5.7; SMP: 6.0; Al^{3+}: 0.0 cmolc dm^{-3}; $H^+ + Al^{3+}$: 4.3 cmolc dm^{-3}; Ca^{2+}: 6.2 cmolc dm^{-3}; Mg^{2+}: 1.4 cmolc dm^{-3}; K^+: 0.22 cmolc dm^{-3}; SB: 7.82 cmolc dm^{-3}; T: 12.12 cmolc dm^{-3}; P: 195 mg dm^{-3}; C: 37.4 g dm^{-3}; V%: 65; Ca/Mg: 4.43. In the 20-40 cm layer, the soil characteristics were: pH CaCl2: 5.6; SMP: 6.0; Al^{3+}: 0.0 cmolc dm^{-3}; $H^+ + Al^{3+}$: 5.0 cmolc dm^{-3}; Ca^{2+}: 4.4 cmolc dm^{-3}; Mg^{2+}: 1.2 cmolc dm^{-3}; K^+: 0.10 cmolc dm^{-3}; SB: 5.7 cmolc dm^{-3}; T: 10.70 cmolc dm^{-3}; P: 61.2 mg dm^{-3}; C: 27.4 g dm^{-3}; V%: 53; Ca/Mg: 3.67.

The planting was carried out using a trellis system with a height of 2.0 meters and two rows of oval wire: one at the top, at 2.0 meters in height, and the other 1.0 meter below it. The spacing between planting rows was 3.0 meters.

For planting, one seedling was used per hole with a spacing of one meter between seedlings, and the average height of the seedlings at planting was 16 cm.

The experimental design used was a randomized block design (RBD) arranged in a 2 x 5 factorial. The treatments represented the combination of two accessions of

M. glomerata Sprengel*.* and five fertilization treatments, including a control. Four replications per treatment were used, with each replication consisting of the average of four plants.

The fertilization treatments consisted of five nitrogen levels: 0, 35, 70, 140, and 280 g per plant, based on the fertilization recommendations for passion fruit cultivation in the states of Rio Grande do Sul and Santa Catarina (SOCIEDADE BRASILEIRA DE CIÊNCIA DO SOLO 2016).

At planting, 35 g per plant of ammonium nitrate (30% N) was applied as a top-dressing, with the remaining doses split into three equal applications at 30, 60, and 90 days after planting. Potassium correction fertilization was applied in the row before planting and incorporated using a rotary hoe.

The experiments were implemented in October 2014 and conducted for periods of 6 and 18 months. Daily irrigations were performed after planting to maintain soil moisture.

After the experimental period, the collected plants were analyzed for the following parameters: dry weight of leaves, branches, and total (g per plant); leaf area (m^2 per plant); and coumarin content (mg per g).

For the determination of leaf area, the disk method was used, according to the studies by HUERTA & ALVIM (1962) and GOMIDE et al. (1977).

To determine the dry weight of leaves and branches, samples representing approximately 10% of the total fresh weight of the plant parts were placed in brown paper bags and dried in a forced-air oven at 65 ± 1°C until constant weight was achieved. The total dry weight values were obtained by summing the dry weight values of leaves and branches.

For the determination of coumarin levels, leaves from the 4th to the 10th nodes from the plant apex were collected. From each leaf, 10 disks with a diameter of 1 cm were cut using a hole punch and then fragmented. The resulting fragments were sifted through a mesh sieve (mesh size 8) with an opening of 2.36 mm. Samples of 0.2 g of the plant material were placed in Falcon tubes containing 25 ml of methanol and then stored in a freezer at -18 ± 1°C for the determination of coumarin content (mg g^{-1}).

The coumarin content in the extracts was determined by high-performance liquid chromatography (HPLC), using equipment consisting of a Shimadzu-LC 10AD pump; LC-Work Station Class LC-10 data system; Shimadzu-SPD 10A UV detector; and CTO10AS oven. The mobile phase consisted of acetonitrile: water (40:60 v/v), with a flow rate of 1.0 mL/min, a column temperature of 40 °C, and detection at 280 nm. The column used was Kinetex LC18, 10 cm x 4.6 mm, 2.6 µm, with a manual injector and a 20 µL injected volume (CELEGHINI et al. 2001)

The quantification of the active principle was performed using coumarin (1,2-benzopyrone) from Sigma-Aldrich® as an external standard. For calibration curve determination, samples with concentrations of 0.00106, 0.00212, 0.00424, 0.00848, and 0.0127 mg/mL were injected in triplicate into the HPLC apparatus. The coumarin content was calculated using the equation derived from the calibration curve, and the result was expressed as a percentage of coumarin.

The nitrogen dose that provided maximum technical efficiency (MTE) was determined by setting the first derivative of the response equation for the variables, which were adjusted using quadratic functions of nitrogen doses, to zero.

The results were subjected to analysis of variance (ANOVA). Initially, the homogeneity of variances among treatments was assessed using Bartlett's test. Variables with homogeneous variances had their treatment effects tested using the F-test. When statistical significance was found ($p \leq 0.05$), the means were further analyzed using polynomial regression via the statistical software Statgraphics Centurion XV Version 15.2.11. The regression models tested were linear and quadratic, with the model selection based on the significance of the regression coefficients.

RESULTS AND DISCUSSION

In all the parameters evaluated, and in both harvest periods, a quadratic effect of nitrogen doses was observed for all the analyzed variables.

The total dry weight values per plant ranged from 274 g to 932 g for accessions G1 and G2, respectively, six months after planting, and from 2056 g to 3700 g at 18 months after planting (Table 1).

Table 1. Total dry mass values (g pl^{-1}) of *M. glomerata* Sprengel accessions grown with different nitrogen doses, 6 and 18 months after planting. Araquari – SC, 2016.

Harvest (months	**access**	**doses of N (g pl^{-1})**				
		35	**70**	**140**	**280**	**Control**
		total dry mass (g $plant^{-1}$)				
6	G1	495.7 b*	544.6 b	485.4 b	274.6 b	354.7 b
18	G1	1959.2 a	2056.0 a	1859.6 a	1017.0 a	633.5 a
6	G2	801.5 b	916.0 b	931.8 b	353.4 b	673.2 b
18	G2	2902.4 a	1881.0 a	370.14 a	2633.8 a	1174.4 a

* Means followed by the same lowercase letter in the column, when comparing the same accession, do not differ by the Tukey test. ($p \leq 0.05$).

This difference in total dry weight values when comparing the averages of plants cultivated for six and eighteen months can be explained by the fact that the harvest conducted at six months after planting occurred shortly after the initial development period of the crop. During this stage, a significant portion of the nitrogen applied to the soil is preferentially directed towards the development of the root system rather than the growth of the aerial parts (CABRAL et al. 2013).

In the harvest conducted eighteen months after planting, the average total dry weight values were higher compared to both the control treatment with no fertilization and the harvest conducted six months after planting. This demonstrates the positive effect of nitrogen fertilization on the dry weight yield in *M. glomerata* Sprengel (Tables 1 and 2).

Table 2. Total dry mass values (g pl^{-1}) of *M. glomerata* Sprengel accessions grown with different nitrogen doses at 18 months after planting. Araquari – SC, 2016.

Access	doses of N (g pl^{-1})				
	35	70	140	280	CONTROL
	total dry mass (g pl^{-1})				
G1	1959.2 a*	2056.0 a	1859.6 a	1017.0 b	633.5 c
G2	2902.4 b	1881.0 c	3701.4 a	2633.8 b	1174.4 d

* Means followed by the same lowercase letter in the column, when comparing the same accession, do not differ by the Tukey test. ($p \leq 0.05$).

The results obtained in the experiment align with those described in the literature by PEREIRA et al. (1998), who evaluated the effect of nitrogen fertilization on biomass production and coumarin content in *M. glomerata* Sprengel. In their experiment, the authors observed a six-fold increase in dry weight values in the nitrogen-fertilized treatment compared to the control treatment.

DUTRA et al. (2016), in an experiment evaluating the effect of nitrogen fertilization on the growth of *Amburana cearensis* Schwacke & Taub seedlings, a medicinal species used for treating respiratory tract diseases and as an anti-inflammatory, observed a positive effect of nitrogen fertilization on the growth parameters of the species.

Increases in the values of aerial dry mass due to nitrogen doses are expected, as the nutrient contributes to vegetative growth by primarily affecting leaf initiation and expansion rates, final leaf size, and stem elongation (SUN et al. 2020).

For the leaf dry weight values, the dose of maximum technical efficiency (MTE) for accession G1, in the six-month cultivation, was achieved with 130 g of pl^{-1}, and for accession G2, it was achieved with 106 g pl^{-1}. In the plants cultivated for eighteen months after planting, the MTE dose for this parameter was 130 g pl^{-1} for accession G1 and 240 g pl^{-1} for accession G2 (Figures 1 and 2).

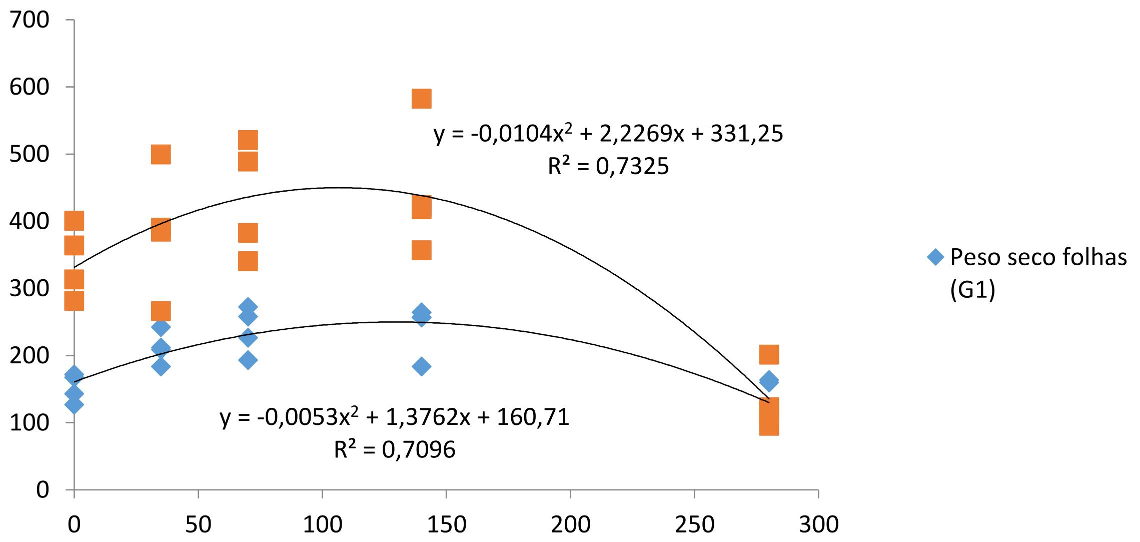

Figure 1. Leaf dry mass (g pl^{-1}) of *M. glomerata* Sprengel accessions subjected to different nitrogen doses and grown for 6 months. Araquari – SC, 2015.

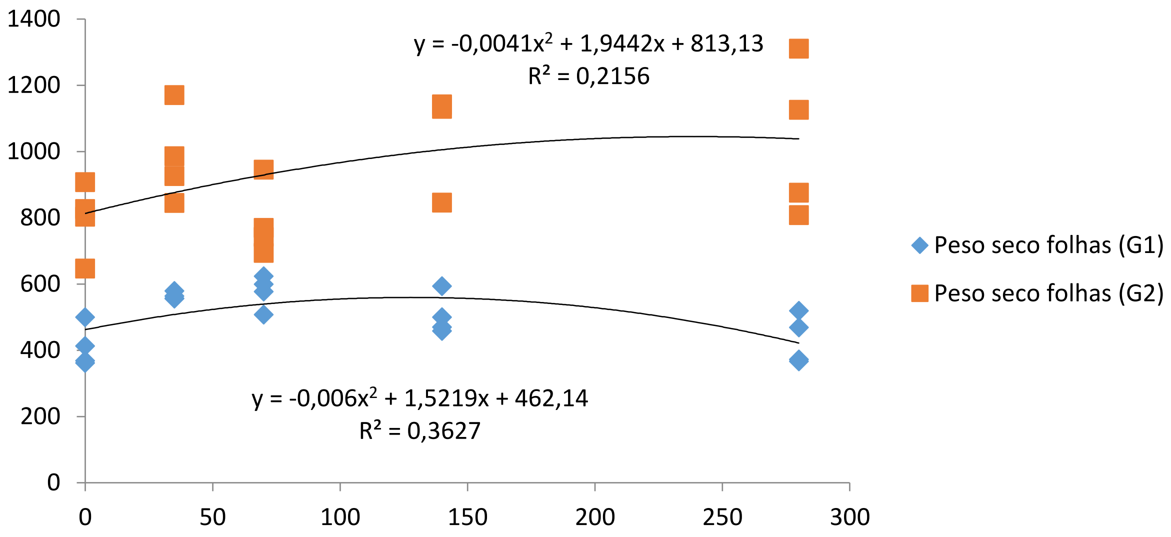

Figure 2. Leaf dry mass (g pl-1) of *M. glomerata* Sprengel accessions subjected to different nitrogen doses and grown for 18 months. Araquari – SC, 2016.

The leaf area values ranged, on average, from 1.87 to 3.52 m² per plant for accession G1 and from 2.22 to 7.43 m² pl-1 for accession G2 in the six-month cultivation. In the eighteen-month cultivation, the values ranged, on average, from 5.0 to 8.9 m² pl-1 for accession G1 and from 11.08 to 13.96 m² pl-1 for accession G2.

The increases in leaf area values between the six-month and eighteen-month cultivations are associated with a higher number of leaves as cultivation time increased. This contributed to an improved light interception area, leading to enhanced carbon assimilation and resulting in positive responses in dry mass values (PEREIRA et al. 2018).

For leaf area values, the dose of maximum technical efficiency (MTE) was achieved with 118 g of N pl-1 for accession G1 and 106 g of N pl-1 for accession G2 in the six-month cultivation. For the eighteen-month cultivation, the MTE doses were 147 g of N pl-1 for accession G1 and 182 g of N pl-1 for accession G2 (Figures 3 and 4).

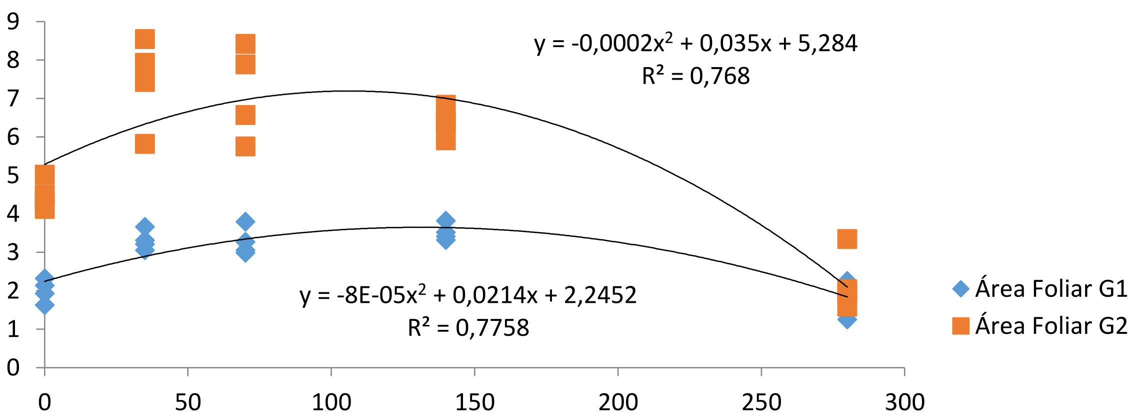

Figure 3. Leaf area (m² pl-1) of *M. glomerata* Sprengel accessions subjected to different nitrogen doses and grown for 6 months. Araquari – SC, 2015.

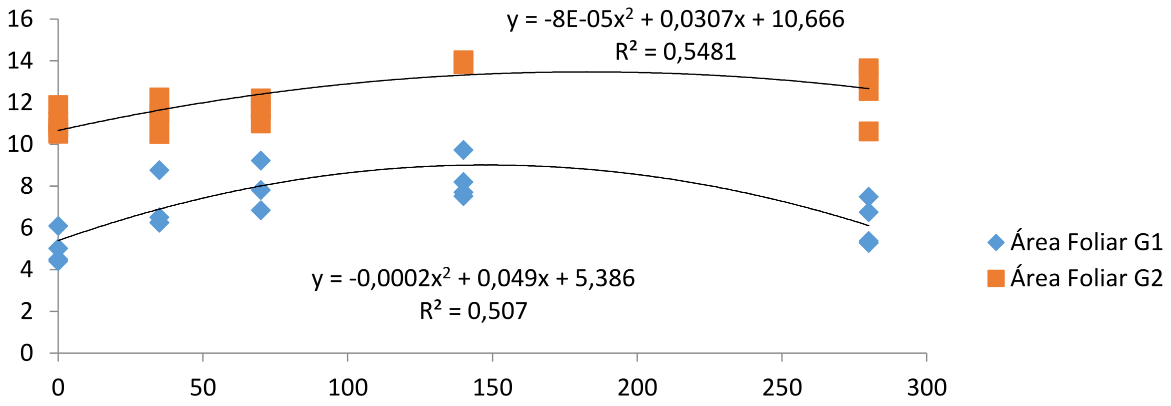

Figure 4. Leaf area (m^2 pl^{-1}) of *M. glomerata* Sprengel accessions subjected to different nitrogen doses and grown for 18 months. Araquari – SC, 2016.

The coumarin content varied from 4.54 mg g^{-1} of fresh tissue in the control treatment with no nitrogen fertilization to 8.59 mg g^{-1} in the treatment with 280 g of N pl^{-1} for accession G1 in plants cultivated for six months. For accession G2, the coumarin content ranged from 1.84 mg g^{-1} to 3.86 mg g^{-1} for the 280 g N per plant dose and the control treatment without nitrogen fertilization (Figure 5).

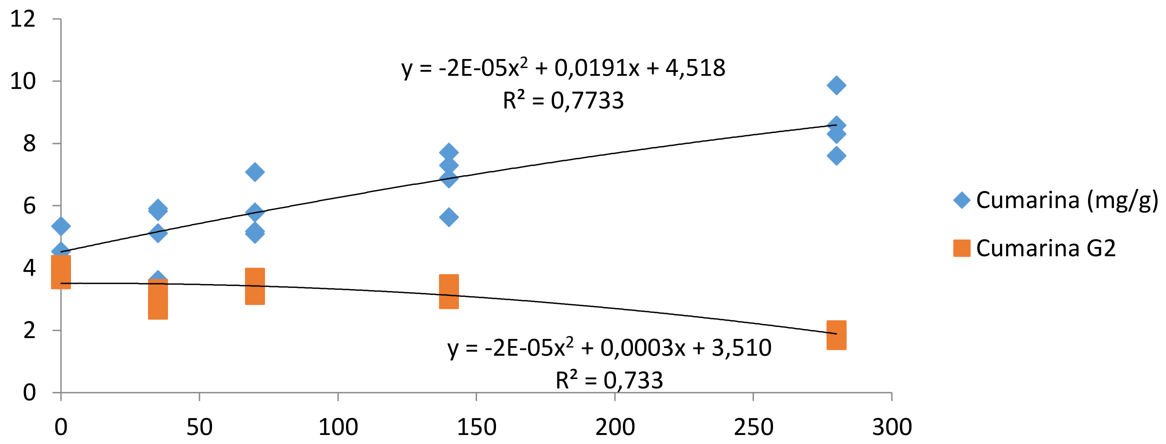

Figure 5. Cumarine content (mg pl^{-1}) of *M. glomerata* Sprengel accessions subjected to different nitrogen doses and grown for 6 months. Araquari – SC, 2015.

Comparing the coumarin contents between the accessions of *M. glomerata* Sprengel in the two cultivation periods, it is observed that the values were higher in plants harvested six months after planting compared to those harvested eighteen months after planting. Specifically, the values were up to ten and six times higher for accessions G1 and G2, respectively, at the dose of 35 g of N pl^{-1} (Table 3).

The first six months of development for the species coincided with the initial sprouting period of the accessions, during which the plants had a large number of very young leaves, despite the low leaf area values (Figure 3).

The large number of young and early-stage leaves may account for the increase in the levels of the active ingredient, as coumarin synthesis occurs preferentially in young leaves and those near the apical bud. This suggests that the meristematic region is the likely site of compound synthesis, likely related to growth and development processes in the plant (CZELUSNIAK et al. 2012).

Considering a population of 4000 plants per hectare and excluding the fertilization treatments, the average dry mass yield of leaves for accessions G1 and G2 in the six-month cultivation were 779 and 1390 kg ha^{-1}, respectively. In contrast, for the eighteen-month cultivation, the yields were 1990 and 3730 kg ha^{-1}.

For the six-month cultivation, the doses of maximum technical efficiency (MTE) for coumarin content were obtained with 587 g of N pl^{-1} for accession G1 and 7 g of N pl^{-1} for accession G2 (Figure 5).

For accession G2, the low nitrogen requirement in the six-month cultivation may be related to the sufficiency of the element provided by the mineralization of the organic matter present in the soil, around 2.7%. This is supported by the fact that the highest coumarin content was observed in the control treatment, with no fertilization. In the six-month harvest, the MTE dose obtained from the first derivative of the regression equation for accession G1 was quite high, which may indicate a greater requirement for nitrogen fertilization for this accession to synthesize the metabolite.

Despite this trend, the application of high doses of nitrogen generally proves to be toxic for plants, interfering with growth processes such as dry matter accumulation. This is because excessive nitrogen can affect the absorption of other essential nutrients for plant development, such as potassium (K), calcium (Ca), and magnesium (Mg) (MARSCHNER 2012).

In the harvest conducted 18 months after planting, the coumarin content varied between 0.46 and 1.69 mg g^{-1} for accession G1, and between 0.52 and 1.52 mg g^{-1} for accession G2, with both variations observed at nitrogen doses of 35 and 70 g N pl^{-1} (Table 3).

The dose of maximum technical efficiency (MET) for the parameter was achieved with 160 g N pl^{-1} for accession G1 and 240 g N pl^{-1} for accession G2, respectively (Figure 6).

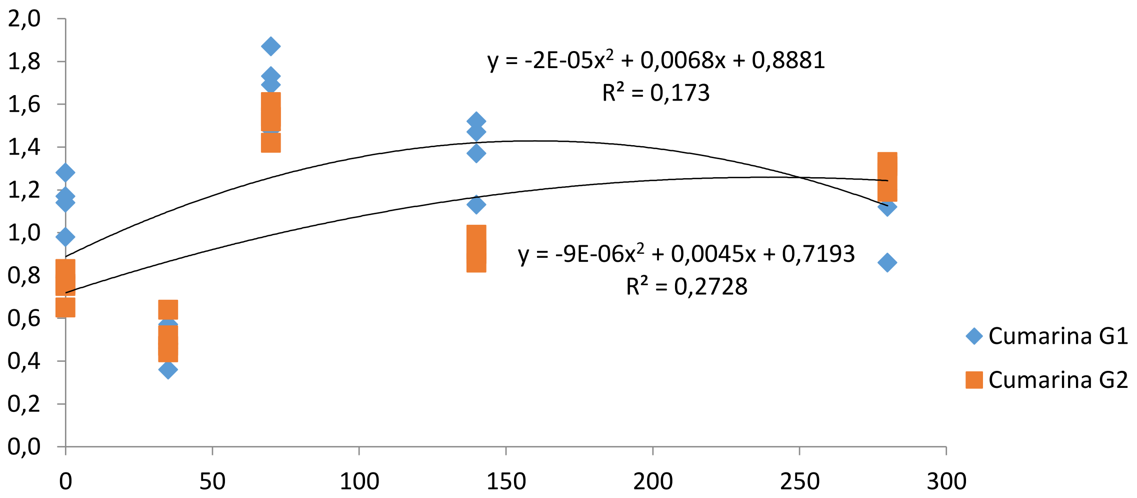

Figure 6. Cumarine content (mg g^{-1}) of *M. glomerata* Sprengel accessions subjected to different nitrogen doses 18 months after planting. Araquari – SC, 2016.

The values of the maximum technical efficiency (MET) doses obtained in the second crop are higher than the doses recommended for passion fruit (*Passiflora edulis* var. flavicarpa Deg.), which were used as a reference for fertilizing *M. glomerata* Sprengel accessions in this experiment and in soils with similar organic matter contents because there is no fertilization recommendation protocol for the species, according to ALVES (2017).

The production of secondary metabolites, such as coumarin in *M. glomerata* Sprengel, is intrinsically linked to biomass production and the accumulation of the active compound in the leaves (MARQUES FONSECA et al. 2021).

Analyzing the data obtained, there is a significant variation in the nitrogen doses that result in maximum technical efficiency (MET) for coumarin levels between different accessions and harvest periods.

For accession G1, the MET doses for coumarin were 587 g N pl^{-1} in the first harvest and 160 g N pl^{-1} in the second harvest. For accession G2, the doses were 7 g N pl^{-1} and 240 g N pl^{-1}, respectively. These values are discrepant, and in some cases, applying high doses, such as 587 g N pl^{-1}, could lead to plant toxicity, corresponding to 2348 kg N ha^{-1} or 104 sacks of urea (45% N).

Thus, for the calculation of coumarin levels, the MET doses obtained for leaf dry mass values in both harvests were adopted: 130 g N pl^{-1} for accession G1 in the first and second harvests, and 106 g and 240 g N pl^{-1} for accession G2 under the same conditions.

Considering the maximum efficiency dose obtained for leaf dry mass productivity and the coumarin content, and taking into account that, according to CORRÊA JÚNIOR et al. (2011), during the drying process of guaco leaves, coumarin levels in the plant tissue decrease by approximately 50% compared to the values obtained for the fresh weight, the following results were obtained: for accession G1, the dry leaf weight per hectare in the first and second harvests were 1020 kg and 2234 kg, respectively. For accession G2, the values were 1819 kg and 4197 kg, with coumarin yields (kg ha^{-1}) of 3.39 and 1.50 for G1 in the first and second harvests, and 3.02 and 2.43 for G2 under the same conditions.

It should be noted that, according to the FARMACOPÉIA BRASILEIRA (2024), the minimum coumarin content for the species should be 0.1% for dried leaves. However, under the conditions described above, the coumarin levels were 0.33% and 0.16% for accessions G1 and G2 in the first harvest, and 0.07% and 0.06% in the second harvest, respectively. In this case, these values are below the minimum recommended by the legislation.

Based on the coumarin production values (kg ha^{-1}) and the percentage of coumarin in the plant tissue, it is recommended to harvest *M. glomerata* Sprengel plants six months after planting, specifically in October, using nitrogen doses of 130 g per plant for accession G1 and 106 g per plant for accession G2.

The correlation analysis between the morphological parameters and the coumarin content (kg ha^{-1}) did not show significant results, with correlation values of -0.01 for total dry weight and 0.13 for leaf area.

CONCLUSION

There was variation in coumarin levels between the nitrogen doses used, demonstrating the effect of the nutrient on the synthesis of the metabolite.

There was variation in the maximum technical efficiency dose between the harvest periods and between the accessions.

Harvesting is recommended six months after planting, using a nitrogen dose of 130 g pl-1 for accession G1 and 106 g pl-1 for accession G2.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and formal analysis**, Luciano Alves and Cícero Deschamps**; software and validation**, Luciano Alves and Cícero Deschamps;** investigation**, Luciano Alves and Cícero Deschamps**; resources and data curation**, Luciano Alves and Cícero Deschamps**; writing-original draft preparation**, Luciano Alves and Cícero Deschamps**; writing-review and editing**, Luciano Alves and Cícero Deschamps**; visualization**, Luciano Alves and Cícero Deschamps**; supervision, **Luciano Alves and Cícero Deschamps**; project administration, **Luciano Alves and Cícero Deschamps**; funding acquisition, **Luciano Alves and Cícero Deschamps**. All authors have read and agreed to the published version of the manuscript.

FUNDING
This work was supported by CAPES - Coordination for the Improvement of Higher Education Personnel

DATA AVAILABILITY STATEMENT
The data can be made available under request.

ACKNOWLEDGEMENTS

The Coordination for the Improvement of Higher Education Personnel (CAPES); Federal University of Paraná; and the Federal Institute Catarinense for providing the resources and infrastructure for the completion of the project.

CONFLICTS OF INTEREST

The authors claim that there is no conflict of interest in the article submitted to the journal.

REFERENCES

ALVES L. 2017. Biomassa e cumarina em acessos de guaco submetidos à redução da radiação ultravioleta e adubação nitrogenada. Tese (Doutorado em Agronomia – Produção Vegetal). Curitiba: UFPR. 110p.

CABRAL CEA et al. 2013. Eficiência de produção e concentração de nitrogênio nos capins marandu, decumbens e convert submetidos à adubação nitrogenada. Bioscience Journal 29: 1653-1663.

CELEGHINI RMS et al. 2001. Extraction and quantitative HPLC analysis of coumarin in hidroalchoolic extracts of Mikania glomerata Sprengel (guaco). Journal of the Brazilian Chemical Society 12: 706-709.

CORRÊA JÚNIOR C et al. 2011. O guaco (*Mikania laevigata* Schultz Bip. Ex Baker), aspectos agronômicos e fitoquímicos. Curitiba: Instituto Emater.

CZELUSNIAK KE et al. 2012. Farmacobotânica, fitoquímica e farmacologia do Guaco: revisão considerando Mikania glomerata Sprengel e Mikania laevigata Schulyz Bip. ex Baker. Revista brasileira de plantas medicinais 14: 400-409.

DA COSTA SOUZA DB et al. 2022. Ação broncodilatadora de *Mikania glomerata* Sprengel (guaco) em pacientes com asma. Revista Farmácia Generalista/Generalist Pharmacy Journal 4: 1-23.

DE MORAIS LAS. 2009. Influência dos fatores abióticos na composição química dos óleos essenciais. Horticultura Brasileira 27: 213-217.

DE MOURA GUERRA AMN et al. 2020. Nitrogênio influencia o acúmulo de biomassa e o rendimento de óleo essencial de manjericão. Brazilian Journal of Development 6: 24739-24756.

DUTRA TR et al. 2016. Crescimento de mudas de umburana (*Amburana cearensis*) em resposta à adubação com nitrogênio e fósforo. Agropecuária Científica no Semi-árido 11: 42-52.

FALCÃO HS et al. 2005. Review of the plants with anti-inflammatory activity studied in Brazil. Revista Brasileira de Farmacognósia 15: 381-391.

FARMACOPÉIA BRASILEIRA. 2024. 7.ed. Disponível em: https://anvisalegis.datalegis.net/action/UrlPublicasAction.php?acao=abrirAtoPublico&num_ato=00000940&sgl_tipo=RDC&sgl_orgao=RDC/DC/ANVISA/MS&vlr_ano=2024&seq_ato=000&cod_modulo=134&cod_menu=1696. Acesso em: 10 fev. 2025.

GIORGI A et al. 2009. Effect of nitrogen starvation on the phenolic metabolism and antioxidant properties of yarrow (*Achillea collina* Becker ex Rchb.). Food Chemistry 114: 204-211.

GOBBO-NETO L & LOPES NP. 2007. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Química Nova 30: 374-381.

GOMIDE MB et al. 1977. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Comparação entre métodos de determinação de área foliar em cafeeiros Mundo Novo e Catuaí. Ciência Prática 1: 118-123.

HUERTA AS & ALVIM PT. 1962. Índice de area foliar y su influencia en la capacidad fotosintetica del cafeto. Cenicafé 13: 75-84.

JONES CG & HARTLEY SE. 1999. A protein competition model of phenolic allocation. Oikos 27: 27–44.

MARQUES FONSECA MC et al. 2021. Biomass production, essential oil's yield and composition of three genotypes of Mikania laevigata Sch. Bip. ex Baker. Acta Physiologiae Plantarum 43: 1-12.
MARSCHNER H. 2012. Mineral Nutrition of Higher Plants. 3.ed. San Diego: Academic Press.
OLIVEIRA LS & SATURINO ASG. 2022. Evidências sobre o uso de Mikania Glomerata Spreng (Guaco) no tratamento de covid-19. Anais do COMED 6: 76-79.
PEREIRA AMP et al. 1998. Influence of fertilizer on coumarin content and biomass production in *Mikania glomerata* Sprengel. Journal of herbs, spices e medicinal plants 6: 29-48.
PEREIRA KA et al. 2018. Parâmetros anatômicos, morfológicos e fisiológicos de forrageiras cultivadas em sistema agrossilvipastoril: uma revisão. Revista em Agronegócio e Meio Ambiente 11: 1333-1355.
RODRIGUES CR et al. 2016. Relação nitrato: amônia na nutrição mineral, crescimento e produção de óleo essencial da Sálvia cultivada em solução nutritiva. Global Science and Technology 9: 43-53.
SOCIEDADE BRASILEIRA DE CIÊNCIA DO SOLO. 2016. Manual de adubação e de calagem para os Estados do Rio Grande do Sul e de Santa Catarina. Comissão de Química e Fertilidade do Solo. 10.ed. Porto Alegre: SBCS.
SOUSA KAG et al. 2023. O uso de Mikania glomerata Spreng.(Guaco) no tratamento de alergia respiratória-uma revisão integrativa. Diversitas Journal 8: 411-420.
SUN et al. 2020. The physiological mechanism underlying root elongation in response to nitrogen deficiency in crop plants. Planta 251: 1-14.
TEÓFILO VN & UHLMANN LAC. 2021. O Uso da Mikania Glomerata no Tratamento Alternativo para Doenças Respiratórias: Revisão de literatura The Use of Mikania Glomerata in Alternative Treatment for Respiratory Diseases: Literature Review. Brazilian Journal of Development 7: 58079-58098.
UENO VA & SAWAYA ACHF. 2017. Influence of temperature on volatile composition of guaco. Holos Environment 1: 125-30.