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Cutting Preference of the Leaf-Heating ant atta sexdens by Seedlings of Eucalyptus urophlylla Submitted to Different Doses of Zinc

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Leaf-cutting ants of the genus *Atta*, also known as leaf-cutting ants, are considered pests in forestry, in various agricultural crops, and also in pastures, causing great damage. Mineral elements are of great importance in increasing crop production and productivity and are involved in the defense mechanism and tolerance of plants to pest and disease attacks, when properly balanced in the soil. The objective of this study was to evaluate the cutting preference of leaf-cutting ants *Atta sexdens* for *Eucalyptus urophylla* seedlings submitted to different doses of zinc (Zn) cultivated in a dystrophic Cerrado Latosol. The doses of zinc provided were 0 (control) and 20 mg/dm³ plus 20 mg/L via foliar, in the form of zinc sulfate. The seedlings used were kept in a greenhouse for up to 80 days and then submitted to laboratory evaluation. The leaves of seedlings treated with different doses of zinc were offered in Petri dishes to three colonies of ants from artificial nests of *A. sexdens* in the laboratory with free choice between treatments. The percentage

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of leaf tissue cut and transported to the nests was evaluated for 20 minutes between different doses and colonies, and four sequential offers of leaves were made. Through the data obtained, it was observed that there was no preference for cutting the leaves of eucalyptus seedlings treated with different doses of zinc by the colonies of leaf-cutting ants *Atta sexdens*.

Keywords: Formicidae; colonies; plant nutrition.

1. INTRODUCTION

Leaf-cutting ants of the genera *Atta* and *Acromyrmex*, also known as saúvas and quenquéns, belong to the main groups of pests of forest species in Brazil. They cause great defoliation in the attacked plants, reduced growth and loss of productivity in the case of wood and, depending on the frequency of the attack, can lead to death, in addition to reducing resistance to the attack of other pests and increasing susceptibility to diseases due to the entry of pests. pathogens for the damage caused [1, 2, 3].

The gains in eucalyptus wood productivity can be affected by *Atta* spp. through the defoliation of the plants and, depending on the severity of the attack and the density of the nests, there may be a drop in wood production by up to 50%. At a density of four adult sauveiros per hectare (three years old), four tons of leaves are consumed, corresponding to 344 eucalyptus trees. An adult sauveiro needs one ton of leaves per year, corresponding to 86 eucalyptus trees [4,5].

Leaf-cutting ants have a high defoliation potential and their control generates a high cost to the producer. Currently, the most used method for ant control is through the use of chemicals. However, as they cause environmental contamination and reach non-target organisms, new management techniques have been sought to help control ants and reduce impacts on the environment [6].

Plant nutrition plays a very important role, as it has a direct effect on the physical and biochemical aspect of the plant. In an attempt to clarify the role of nutrients in plant metabolism and in resistance to pest and disease attack, studies have been carried out on the relationship between nutritional balance and its beneficial effect on plants in terms of pest attack. One of the main theories currently known is the trophobiosis theory, which tells about the nutrition effect and its relationship with insect attacks [7, 8, 9]. Zinc is responsible for the biosynthesis of tryptophan, which is a precursor of the phytohormone indoleacetic acid, which acts directly on cell division and plant growth, increasing the efficiency of water and nutrients. Zinc-deficient eucalyptus plants often show stunting and the leaves are small and clumped. In *E. urophylla*, leaves that are still growing initially show interveinal chlorosis, and areas with a purplish color may appear and with the worsening of the deficiency, the leaves can reduce in size and the internodes shorten, leaf tips and spots on the interveinal tissue can become if necrotic [7, 10].

According to the theory, the nutritional status of the plant can determine the resistance or susceptibility to attack by pests and pathogens. Resistance can be increased through changes in leaf anatomy (thicker epidermal cells and greater degree of lignification or silicification) and changes in physiological and biochemical properties, with greater production of repellent or inhibitory substances [11].

Zinc is a nutrient that has reports in the literature on the control of the leaf-cutting ant symbiont fungus Atta sexdens, in addition to other reports on the reduction of the stalk borer attack in corn with adequate zinc nutrition to the plants.

Perennial woody species direct more energy in the production of defense compounds, known as secondary substances, which have the function of increasing the plant's defense capacity against pests and diseases. modified when they undergo water or nutritional stress (lack or excess). The nutritional status of the plant and its resistance to pest attack vary according to the type and amount of the nutrient to be considered [12, 13, 14].

Leaf-cutting ants forage through environmental stimuli, such as the odors exhaled by plants through volatile substances, which are captured by worker ants and intercepted by antennae. Although there is no precise explanation of how the selection of vegetables by leaf-cutting ants occurs, studies cite the choice for nutritive quality for the insects or the symbiont fungus that they feed on, in addition to citing the chemical defense substances contained in the plants as well as its water content [15, 16; 17].

Therefore, the objective of this work was to evaluate the cutting preference of leaf-cutting ants *Atta sexdens* Linnaeus, 1758 (Hymenoptera: Formicidae) by *Eucalyptus urophylla* seedlings submitted to different zinc doses.

2. MATERIALS AND METHODS

2.1 Seedling Production

The seedlings were produced in a small rural property, located in the municipality of Paraíso do Tocantins - TO, and the evaluation of the cut preference was carried out in the laboratory of Insect-Microorganism Symbiosis, Federal University of Tocantins, Campus de Gurupi, in the period of May. from 2021 to July 2021. The soil for the production of the seedling substrate was collected in the deepest layers, 20 to 40 cm, classified as dystrophic Red Latosol, following the Brazilian Soil Classification System (SiBCS), with latitude coordinates 10°6 '30.0222" and longitude 48°55'33.82896".

From the collected soil, a sample of 300 grams was taken for chemical analysis and, following the methodology of the Embrapa Clima Temperado - RS Soil Fertility Laboratory, it was placed in a ventilated place in the shade and later, packed in a transparent plastic bag, identified and sent to the Safrar Análises Agrícolas laboratory, located in the municipality of Patrocínio - MG and the following results were obtained, shown in Table 1. After the chemical analysis of the soil and for the composition of the substrate, 50% of the substrate volume of Cerrado soil, 30% of well-tanned bovine manure and 20% of sand were used, following the recommendation of Embrapa Cerrado for the production of eucalyptus seedlings. (1997) and to adapt the soil according to the nutritional requirements of eucalyptus seedlings, the recommendations of correctives and fertilizers for Goiás 5th approach (1988) were followed.

The seeds of the E. urophylla species were acquired from the company NC Aromas, located in the city of São João do Paraíso - MG, and were sown in polyethylene forest trays with the substrate produced. The zinc source used was zinc sulfate (ZnSO4) and all adjustments were made in relation to the other nutrients required for the production of eucalyptus seedlings, following the chemical analysis of the soil. The sources of the other necessary nutrients were: simple superphosphate, potassium chloride, urea, boric acid and dolomitic limestone to supply calcium and magnesium to the seedlings. For a homogeneous distribution of nutrients in the substrate, all sources of nutrients were diluted in water and subsequently applied to the substrate.

After sowing, the trays were placed in a cage with a mesh to protect against insects, Fig. 1 (B), in order to prevent the attack of the gall wasp *Leptocybe invasa* Fisher & La Salle (2004) (Hymenoptera: Eulophiidae) and to avoid possible interferences. in the results obtained. Seedlings were produced without zinc application and with zinc application via soil plus foliar, being a dose of 20 mg via zinc soil plus 20 mg via foliar, in the choice of doses and to avoid possible intoxications, the methodology applied by Rodrigues et al. [18].



Fig. 1. Eucalyptus urophylla seedlings housed in a cage with insect protection mesh, A and B

pH (H ₂ O) ⁽¹⁾	Ca ²⁺⁽²⁾		Mg ²⁺⁽²⁾	Al ³⁺⁽²⁾	H+AI ⁽³⁾	T ⁽⁷⁾	T ⁽⁷⁾	V ⁽⁷⁾	m ⁽⁷⁾
	cmolc dm ³							%	
5,0	0,60		0,30	0,00	4,70	5,7	1,00	17,50	0,00
P meh. ⁽⁴⁾	K ⁺⁽⁴⁾	Fe ⁽⁵⁾	Cu ⁽⁵⁾	Mn ⁽⁵⁾	Zn ⁽⁵⁾		B ⁽⁵⁾	M.O ⁽⁶⁾	
mg dm ³								dag kg	
1,7	38,00	18	0,6	2,9	0,7		0,32	1,70	

Table 1. Chemical characteristics of the soil collected in the area

pH in water⁽¹⁾, ⁽²⁾ Ca²⁺, Mg²⁺ e Al³⁺: extractor KCl 1 mol L⁻¹, ⁽³⁾ H+Al: Buffer Solution SMP at pH 7,5, P e K^{+ (4)}: extractor Mehlich-1, B: extractor BaCl2 . 2H2 O 0,125% the hot, ⁽⁵⁾Cu,Fe,Mn,Zn: extractor: DTPA in pH 7.3, ⁽⁶⁾M.O.: colorimetric method, T: CTC pH 7,0, t: CTC effective, V%: base saturation, m%: aluminum saturation



Fig. 2. Offer of leaves of Eucalyptus urophylla seedlings that did not receive zinc and that received a dose of 20 mg/dm³ plus 20 mg/L via foliar to the colonies of leaf-cutting ants, A and B

The application of the dose via foliar was applied in three weekly installments after 45 days of emergence of the seedlings and according to the development of the leaf area, with the first dose constituting 20% of the total dose to be applied, the second dose 30% and the third dose of 50%, thus constituting 100% of the dose evaluated in the experiment. For pulverized application, the dilution of zinc sulfate was carried out in water and to improve the efficiency of application of the solution, vegetable oil was used, in view of the high waxiness contained in the surface of the leaves of the eucalyptus seedlings.

The application of zinc via foliar aimed to increase the zinc content in the leaves of eucalyptus seedlings, considering that the foraging of leaf-cutting ants is dependent on several factors and changes in the chemical composition of the leaves could directly influence this process.

2.2 Assessment of Cutting Preference by Leaf-Cutting Ants

The seedlings were raised in the nursery until 80 days old and then submitted to the cutting preference test by leaf-cutting ants at the Insect-Microorganism Symbiosis Laboratory, Universidade Federal do Tocantins, Campus de Gurupi, Tocantins, Brazil.

To evaluate the cutting preference, three colonies of A. sexdens kept in the laboratory with controlled temperature, relative humidity and photoperiod were used. The ants are fed with leaves of *Mangifera indica L.* (mango), *Anacardium occidentale L.* (cashew), Citrus sp., as well as oat flakes and wheat bran. Before offering the leaves of Eucalyptus urophylla seedlings, the leaf-cutting ants were not fed for 24 hours, avoiding possible interference with foraging.

Petri dishes were used to offer the leaves, where the leaves were placed and left inside the colonies to observe the selection and preference for cutting the materials offered, performing four replications. In this case, two to three leaves were placed in petri dishes within each colony, both with seedling leaves without zinc application and with zinc application of 20 mg/dm³ via soil plus 20 mg/L via foliar, following some procedures of the methodology applied by Marsaro Júrior et al. [19], Fig. 2. As they were seedling leaves, there was no choice for offering in relation to leaves from the lower, middle and upper thirds.

The test of attractiveness and courtship preference was carried out in two periods of the day, from 10:00 am to 12:00 pm and from 2:00 pm to 4:00 pm for two days.

The percentage of cutting and loading of leaf fragments cut for a period of 20 minutes, the time required for cutting and total leaf loading of the different treatments used in the evaluation, were evaluated. For the application of this methodology, tests of offer were carried out with the leaves of the eucalyptus seedlings before starting the evaluations, with the objective of defining the ideal time for the choice of material, cutting and total loading of the leaves to the nest.

After the elapsed time, the leaves that remained in the petri dishes were weighed, and the percentage of mass consumed by each colony was calculated by difference. During the offer tests, the seedlings were kept in the same conditions of environment and inside the laboratory, but without access to the ants. The data obtained were submitted to analysis of variance and the means were compared by the Tukey test at the 5% probability level using the Sisvar software version 5.8.

3. RESULTS AND DISCUSSION

The evaluations carried out showed that there was no significant difference in foraging leaves of *Eucalyptus urophylla* seedlings treated with different doses of zinc by *A. sexdens* colonies, that is, the dose of 20 mg/dm³ plus 20 mg/L did not cause inhibition of foraging. During the offer of leaves, the leaf-cutting ants selected, cut and carried the fractionated material to their respective nests without any preference, Table 2.

Table 2. Average percentage of leaf foraging
of Eucalyptus urophylla seedlings treated
with different doses of zinc performed by A.
sexdens colonies

Doses	Foraging
0	44,25 a
20	57,08 a
Média	50,66
CV%	52,61

Note: Means followed by the same letter in the column do not differ statistically from each other by Tukey's test (p>0.05%) Even though there was no significant difference in foraging of leaves from seedlings treated with different doses of zinc, the graph in Fig. 3 shows that while colony 2 did not have a preference in foraging leaves treated with different doses, colony 1 and 3 showed a percentage of foraging of 62 and 64.5% when the leaves offered were from seedlings that did not receive doses of zinc and of 0 and 32.75% when the leaves were from eucalyptus seedlings treated with a dose of 20 mg/dm³ via soil more 20 mg/L via foliar zinc, indicating that there was a lower percentage of foraging at the highest dose of zinc.

According to Schlindwein [20], the behavior during foraging of leaf-cutting ants is a complex process and most of the time, for better understanding, it is necessary to study the individual, the colony or both.

Holldobler & Wilson [21] reported that each leafcutting ant species has a foraging schedule and, depending on the distance they need to travel in search of food, they can be more selective in relation to this food, and the further away from the food, the, the greater the selectivity. Also according to the authors, the leaf-cutting ant species *Atta sexdens rubropilosa*, in the cutting activity, the distance from the food to the nest may be the most important factor.

According to Schlindwein [20], plants with greater preference and with greater distance from the nests were cut with less intensity in relation to plants that were close to the nest and the scouts. In the experiment evaluated, the distance did not influence the selectivity, considering that the leaves were offered within the colony. The selectivity of the material to be cut can occur through the presence of toxic compounds or changes in the nutritive value required by the antagonist fungus and also by the physical and mechanical properties of the plants [22].

Silva [23], evaluating the effect of the fungus *Trichoderma harzianum* Rifai, (1969), and zinc on colonies of A. sexdens, observed that the supply of zinc, using zinc chloride and zinc sulfate as a source, at the concentrations of 2.5 and 5.0 g/L in Petri dishes in PDA medium for the development test of the symbiotic fungus *Leucoagaricus gongylophorus* (Singer) Moller inhibited its growth.

Although several factors can explain the selectivity of the ants in relation to the food resource to be cut and taken to the nest, mainly in relation to toxic compounds and changes in the nutritional value required by the symbiont fungus, in the work carried out, selection and preference was not observed. of cutting of eucalyptus leaves by the ants *A. sexdens* in relation to the concentrations of zinc applied to the seedlings both via soil and foliar, in view of the reports of toxicity and inhibition of fungus arowth.

In the application of the trophobiosis theory in the selectivity of leaf-cutting ants in relation to the material to be cut, there are several studies that report the cutting preference of leaf-cutting ants in relation to the supply or absence of certain nutrients to the plants.





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Nascimento [24] in a study evaluating the effect of nutrient restriction on the development of teak seedlings and the preference for cutting by leafcutting ants of the species *A. sexdens* and *Atta laevigata* (F. Smith, 1858), observed that there was a preference for cutting in the treatment without the addition of phosphorus, followed by treatments with the omission of magnesium, calcium, potassium and silicon. The author observed that there was less transport of leaf fragments offered when the treatment was complete, that is, with all the nutrients required by the crop. In addition, he observed that the control and without nitrogen addition also had a lower cut preference.

Ortiz et al. [[25], verifying the foraging response of *Acromyrmex rugosus* (Smith, 1858) and *Acromyrmex balzani* (Emery, 1890) to seedlings of *Eucalyptus camaldulensis* (Dehnh., 1832) with different nutritional restrictions, found that leaf discs offered to leaf-cutting ants with treatment in the absence of phosphorus showed the highest preference for cutting, followed in descending order by treatments with the absence of potassium, calcium and nitrogen.

According to Marschner [26], mineral nutrition can contribute to the level of plant resistance to attack by pests or diseases through histological or morphological changes and also in chemical composition.

Although no preference was observed in foraging of leaves treated with different doses of zinc, the literature reports that high doses of zinc can reduce the levels of amines and amino acids in young leaves, thus interfering with insect attack. Zinc deficiency causes a reduction in IAA (indoleacetic acid) and RNA, lower production of phenols and lignin [27, 28].

Leaf-cutting ants have the ability to chemically distinguish new leaves from old leaves, with a preference for new leaves [29]. During the supply of eucalyptus leaves to the colonies of leafcutting ants, there was no choice of new or old leaves from the seedlings, and both new and old leaves were offered to the ants.

According to Ribeiro & Marinho [16], leaf-cutting ants of the genus *Atta* have a complex behavior in relation to the choice of food resource and the collection of cut materials for the cultivation of the fungus. And according to Endringer (2015) [30], in the same colony in the laboratory, the behavior in choosing the material to be cut can be changed. The results found during the evaluation and the complex foraging behavior of leaf-cutting ants evidence the need for continuity of studies evaluating the most diverse factors that interfere in the preference of the material and the effect of zinc on the attractiveness of the ants.

4. CONCLUSION

There was no preference for cutting by the leafcutting ants *A. sexdens* for the leaves of *Eucalyptus urophylla* seedlings treated with different doses of zinc. precise in relation to the effect of zinc on the foraging of leaf-cutting ants *A. sexdens*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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