Original Research Article

Plants mineral nutrition on the preference of *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) in *Eucalyptus* sp.

ABSTRACT

The plants nutritional maintenance is a support to their natural defense in relation to the insects attack of economic importance. The mineral nutrition arises as an alternative, since it provides the plants silvicultural conditions necessary to express all their productive potential, with the power of resistance to biotic constraints. Thus this study had as objective to evaluate the effect of different formulations of mineral fertilization in the eucalyptus plantations in relation to occurrence of *Glycaspis brimblecombei* (Moore), in the field. The experiment was carried out in the field from December 2015 to December 2016. The experimental arrangement was planned provided in the factorial design, with three origins of *Eucalyptus* sp. (*Eucalyptus camaldulensis* Dehn; hybrids *(Eucalyptus urophylla* S.T. Blake) x *Eucalyptus grandis* (Hill ex Maiden) and *E. urophylla* x *E. camaldulensis*); and seven nutritional formulations. The research data were subjected to analysis of variance (ANOVA), under a randomized block design in a factorial scheme (3 x 7), and the means compared by the Tukey test at 5% probability of error. The response variable used in the analysis was the occurrence of eggs and lerps, being transformed, in order to become suitable for parametric tests. The population fluctuation was carried out in the planting annual cycle. For the correlation analysis between the dependent and the independent variables, the data were analyzed by Pearson correlation (r) and their values were analyzed by using the Student's T-Test. The omissions influenced on the preference of the red gum lerp psyllid to *Eucalyptus* sp. In *E. camaldulensis* the omission of nitrogen and potassium influence on the preference and the absence of silicon causes the favoring of the pest oviposition. In the hybrid *E. urophylla* x *E. grandis* the absence of fertilization and the absence of nitrogen, phosphorus, potassium, individually, are less preferred.

Keywords: resistance, forestry, suckers, red gum lerp psyllid, hybrids, natural defense, plant nutrition, economic importance.

1. INTRODUCTION

Forest species are of great importance to the country's economy, as they provide a range of commercial products. The eucalyptus cultivation occupies approximately 7.41 million hectares of planted forests in the country. The value of forest production increased by 3.4% to R$ 19.1 billion. Of this amount, R$ 14.8 billion, or 77.3%, refers to forestry, an increase of 5% over 2016. Timber products account for 90% of the value of the country's forest production and have increased by 3.6% last year. Separated into categories, wood products planted for commercial purposes increased by 5%. According to the IBGE, this is due to the greater control in the exploitation of the native species and the incentive to the preservation of the forests [1].

Originated in Australia, *Eucalyptus* is host of many species of insects of the Family Aphalaridae [2]. Among these, the red gum lerp psyllid is considered a specific *Eucalyptus* pest, with great potential for destruction due to ease of dispersion [3]. The damages can be indirect, serving as viruses vectors; or direct, sucking the sap and introducing into the trees toxic substances through their saliva [4]. Below the protective layer of this insect, identified as lerp

(excreta composed of waxes and sugars) the nymphs develop until adulthood, passing through five instars of development, in this period, their intense sucking of sap, cause the trees defoliation, which may reach their entirety according to the level of infestation, it is worth mentioning that the intense defoliation causes a significant reduction in forests productivity, and when there are successive annual attacks there is the consequent plant death. The quantification of the damages, under economic aspect, must be carried out because the literature is scarce and nonexistent in some countries with in Brazil.

The life cycle of the red gum lerp psyllid takes from 1 to 2 months, depending on the regional temperature [4], which can be worrying for the cultivation in the Center Western Brazil. Due to the rapid dispersal and establishment the pest eradication methods are considered effective, expensive and inefficient [5].

The plants nutritional status can affect the ability to pests attack support [6], after all, the host quality is key to the insects establishment and development, as well as the nutrition can change the damages intensity, the potential and the reproduction strategies of these individuals [7; 8], as well as the insects control of economic importance. The relationship between the host nutrition and the insects may indicate the need for certain element favoring the occurrence or not of the forests plantation attack.

The nutritional balance will promote the appropriate metabolic development to the plant, without the presence of free radicals, according to the trofobiose theory, a plant can only be attacked by pests when the food necessary to the same is found in the plant sap. The excess or lack of a nutrient can lead to deficiency of others, with immediate reflex in the metabolism, and this imbalance causes the predominance of the proteolysis process (proteins degradation) in the tissues [6], making soluble substances available, readily accessible for the insect's nutrition.

In optimal development conditions the plants are capable of carrying out physiological and morphological changes in response to extrinsic stimuli, when these changes reduce the survival, reproduction or preference of the herbivore on the plant, there is the induced resistance [9]. This plants capacity is associated to protein synthesis, which may be affected by various biotic and abiotic factors. Thus, this research aims at evaluating the effect of different formulations of mineral fertilization in the eucalyptus plantations concerning the occurrence of *Glycaspis brimblecombei* (Moore), in the field.

2. MATERIAL AND METHODS

The experiment was installed in the field, in Fazenda Mato Grosso of private property, located in the municipality of Juscimeira/MT, under the geographical coordinates 15°57'07"S latitude and 54°32'42" E longitude with an altitude of 504 m.a.l.s. The experimental area soil is classified as a dystrophic Yellow Latosol of sandy texture, according to Embrapa, [10]. For the soil analysis the samples were collected at a depth from 0.20 to 0.40 m.

The experimental design was planned in a factorial scheme, with three origins of *Eucalyptus* sp. (*E. camaldulensis*; and the hybrids *E. urophylla* x *E. camaldulensis* ("urocam") and *E. grandis* x *E. urophylla* ("urograndis"); in seven formulations of fertilization, in a total of 21 treatments arranged in four blocks. The seedlings were arranged in space 3 m x 3 m, in a total of 15 per plot, with the three measurable central seedlings, and the other as a simple border.

The used treatments were: T1: conventional fertilization (N = 160 kg, P_2O_5 = 60 kg, K_2O = 180 kg, Si = 2.5 mL L^{-1}, FTE BR12 = 50 kg, Correction of soil pH (0.5 T of dolomitic limestone with PRNT of 92 % + 0.5 T of gypsum). T2: conventional fertilization without addition of N T3: conventional fertilization without adding P. T4: conventional fertilization without addition of K. T5: conventional fertilization without addition of silicon (Si). T6: conventional fertilization without pH correction. T7: only pH correction.

In the area preparation the correction of soil pH was performed with application of lime and gypsum, 90 days prior to the experiment installation, being the experiment performed in the period from December 2015 to December 2016. The treatments were provided differently, P and the micronutrients in total quantity in planting fertilization , N and K split in planting fertilization and three more top dressing fertilization (quarterly), with four four-monthly foliar Si applications.

With the detection of red gum lerp psyllid in natural infestation in the study area, the population fluctuation was carried out in the planting annual cycle, with fortnightly collections for the eggs and nymphs monitoring. Leaves were collected at three different heights per tree, for each sample plot, then they were placed inside plastic bags, properly identified, and then sent

to the Laboratory of Forest Protection, at Faculdade de Engenharia Florestal/UFMT, for screening and quantification of insects.

The research data were subjected to analysis of variance (ANOVA), the experiment was analyzed in a randomized block design in a factorial scheme (3 x 7), and based on the same it was verified that significant differences exist; the means were compared by the Tukey test at 5% probability of error. The response variable used in the analysis was the occurrence of eggs and lerps collected in leaves samples, these being transformed by the formula *log(x+1)*, where x is the value of each variable, in order to become suitable for parametric tests.

For the fluctuations charts the biweekly means of the variables quantities of eggs and lerps collected in each origin of eucalyptus were summed and presented in the corresponding months, in relation to the weather data, such as: temperature (ºF); moisture (%); and precipitation (mm) which were provided by the website of the National Institute of Meteorology in conventional station of Poxoréo-MT, OMM code: 83358.

To detect if there is a correlation between the dependent variables (lerps and eggs) with the independent ones (origins of eucalyptus) data were analyzed by Pearson correlation (r) and their values were analyzed by using the Student's T-Test for reporting the significance of correlation. The r values were based on: from 0.0 to 0.2 zero correlation; 0.21 to 0.40 weak correlation; 0.41 to 0.70 substantial correlation; 0.71 to 0.90 strong correlation; and 0.91 to 1.0 as extremely strong correlations [11].

3. RESULTS AND DISCUSSION

The according to the result of soil analysis, the soil has the following physical chemical characteristics (Table 1).

Table 1. The result of soil analysis with its following physical chemical characteristics.

pH ($CaCl_2$)	**O.M**	**P** (Mehlich)	**K**	**S**	**Al^{+3}**	**$H^{++}Al^{+3}$**	**Ca**	**Mg**	**SB**
	g kg^{-1}	---------- mg dm^{-3} ----------			---------- $cmol_c$ dm^{-3} ----------				
4.5	9.9	1.9	14	6	0.2	2.3	0.5	0.3	0.8
CEC	**B**	**Cu**	**Fe**	**Mn**	**Zn**	**V%**	**Sand**	**Silt**	**Clay**
	---------- mg dm^{-3} ----------				**%**		---------- g kg^{-1} ----------		
3.1	0.09	0.4	97	24.7	0.1	26.8	78	37	885

O.M = organic matter; P = phosphorus; K = potassium; S = sulfur; Al^{+3} = aluminum; Ca = calcium; Mg = magnesium; SB = sum of bases; CEC = cation exchange capacity; B = boron; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc; V% = base saturation

In the egg-laying the preference of the red gum lerp psyllid for *E. camaldulensis* was evident (65% of 74,701 eggs and 60% of 39,547 lerps out of the total sampled), when this eucalyptus was compared to other hybrid eucalyptus of the experiment, even with the action of different formulations of fertilization (Table 2).

Table 2. Totals values of screening of *Glycaspis brimblecombei* (Moore) during the evaluation year in a commercial plantation with different *Eucalyptus* in Juscimeira, MT (2016).

	Treatments	***Eucalyptus***					
		E. camaldulensis		"urocam"		"urograndis"	
		Eggs	Lerps	Eggs	Lerps	Eggs	Lerps
1	Conventional	8,999	6,314	1,749	997	1,639	1,156
2	Without N addition	4,655	3,357	2,366	1,454	1,301	653
3	Without P addition	6,334	3,063	3,322	1,060	885	742
4	Without K addition	3,694	2,065	2,840	1,982	706	548
5	Without Si addition	13,778	2,907	3,010	1,621	1,346	845
6	Without correction	6,203	2,362	2,247	1,690	2,306	1,182
7	Without fertilization	5,246	3,852	1,235	992	840	705
	Total	**48,909**	**23,920**	**16,769**	**9,796**	**9,023**	**5,831**

The hybrids, however, with lower percentages of infestation, to "urocam" egg count with 22% and "urograndis" with 12%, to urocam lerps 25% and "urograndis" 15%. Many are the studies that indicate *E. camaldulensis* as the most susceptible species to red gum lerp psyllid attack, since it allows its full development from the eggs stage until adulthood [12].

Regarding the egg-laying, as observed in Table 3 the treatments that differed among themselves and that significantly influenced the species *E. camaldulensis* were the treatments 4 and 5, respectively, absence of K with the lowest mean and without addition of Si with the highest observed mean among the other eucalyptus and their respective treatments.

Table 3. Factorial analysis of sampling of *Glycaspis brimblecombei* (Moore) during the evaluation year in a commercial plantation with different *Eucalyptus* in Juscimeira, MT (2016).

Treatment	*Eucalyptus*											
	E. camaldulensis				“urocam”				“urograndis”			
	Eggs		Lerps		Eggs		Lerps		Eggs		Lerps	
Conventional	2250	abA	1579	aA	437	aB	249	aB	410	abB	289	abB
Without N addition	1164	abA	839	abA	592	aAB	364	aB	325	abB	163	bcC
Without P addition	1584	abA	766	abA	831	aA	265	aB	221	bB	186	abcC
Without K addition	924	bA	516	bA	710	aA	496	aA	177	bB	137	cB
Without Si addition	3445	aA	727	abA	735	aB	405	aAB	337	abB	211	abcB
Without correction	1551	abA	591	bA	562	aB	423	aAB	577	aB	296	aB
Without fertilization	1312	abA	963	abA	309	aB	248	aAB	210	bB	176	abcB
Eggs: *p value* = 0.0062**; cv = 6.97%							Lerps: *p value* = 0.0007**; cv = 6.56%					

**Significant at 1% probability of error; means followed by the same letter in columns and capitalized on the lines did not differ statistically among themselves at 5% probability by Tukey test.

The proteins and amino acids are primary sources of insects demand, and are associated directly to the eggs production in adult insects [6]. The Si application influences in the highest mortality of nymphs of the red gum lerp psyllid causing a significant reduction in the decrease of infestation of *G. brimblecombei* [13], because according to Epstein, [14], Si has the function of reinforcing the cell wall and thus increase the resistance against pathogens and insects.

In hybrids, the preference for individuals to "urograndis" was not influenced by the treatments, without the soil pH correction, where the average sampled quantity was larger than the others, and the treatments 3, 4 and 7, significantly equal, showed the lowest oviposition means. The fertilization procedure most of times has the function to infer in the crops growth, but this action alters the levels of nutrients in different parts of the plant and consequently in the insect populations. Among the main functions of Ca, it is emphasized herein, the action of this element in the permeability and structuring of the plants cell wall [15], fact which corroborates in the plant resistance to the procedure of the psyllid oviposition.

In "urocam" there was no significant difference in any of the stages of the evaluated development. The lack of connection between preference and performance of *G. brimblecombei* in different eucalyptus clones can be related to the fact that this insect is out of its area of natural occurrence, in recent contact with hybrids produced in Brazil. Therefore, the red gum lerp psyllid did not have still enough time to adapt to the chemical and/or physical pathways that would indicate to females which are the best oviposition sites. [16].

The nymphal stage (lerps) of psyllids was a reflection of the nitrogen management, where for *E. camaldulensis* treatments without K and without soil pH correction resulted in lower mean quantities, in distinction to treatment with conventional fertilization that had the highest mean in this experiment. The mineral formulations recommended for fertilization, are usually based on the adequacy of the average of studies of the genus, without considering the individual needs of each forest species, therefore, the possible imbalance in the plant may cause the attractiveness and preference of the red gum lerp psyllid to the potential host.

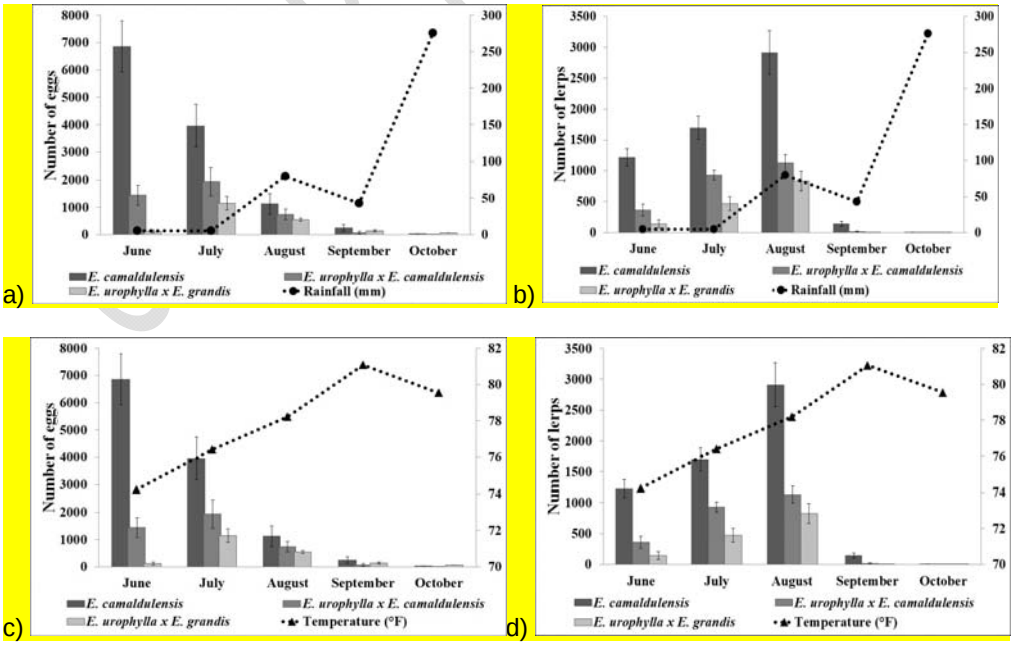
The nutritional imbalance in N/K ratio is one of the factors of greatest concern to the plants protection, because the lack of K results in the accumulation of substances of lower molecular weight and the increase of soluble nitrogen compounds; however, the excessive N causes the proportion to be higher, generating the host attractiveness. However, when the psyllid feeds from high levels of N its nymphal period decreases and increases its longevity in adult individuals, i.e., higher reproductive potential, more time for mating and increase in the number of layings [13].

The presence of lerps in the hybrid "urograndis" occurred differently to the fertilizations influence, the treatment without soil pH correction resulted in a higher incidence of nymphs development. The inadequate supply of Ca and Mg results in imbalances, which may induce nutritional deficiency and opportunity to pests attack [17], taking into consideration the importance of the Ca functions (translocation of carbohydrates and nutrients) and Mg (protein synthesis) for the plants. The soil acidity may have affected the nutrients release for the roots absorption, in addition to not providing Ca to the trees, considering the importance of this element to the forest species [18].

The treatment without addition of K had the lowest mean count of lerps of the experiment, which was justified because, in their absence there is no osmotic adjustment of the plant cells [15] occurring less sap flow in the plant, therefore, the nymphs of the psyllid cannot complete their development cycle in the host. However, it can be concluded that regarding N/K ratio the dose of N in the formulation was adequate to the K natural of the soil for the planting of the hybrid "urograndis".

The weather variations interfere in the insects biology, in the case of red gum lerp psyllids, indirectly in the oviposition, since they influence the population of adult individuals, and consequently the quantity of eggs and lerps, and directly on the lerps development, because these are composed mainly of sugars, that with action of water, temperature and moisture are removed from the leaves where they grow. According to Tuller et al., [19] the occurrence of red gum lerp psyllid responds to climatic conditions, and may directly interfere in the number of individuals. According to Firmino-Winckler, et al., [4], the growth of the population of *G. brimblecombei* is inversely proportional to the temperature, reaching higher peaks of infestation in the winter months.

In all the eucalyptus the presence of red gum lerp psyllid were observed during the drought period. The samples showed oviposition peak in the month of June in *E. camaldulensis* and July for the hybrids “urocam" and "urograndis"; and higher presence of nymphs in the month of August at all eucalyptus (Fig 1). Silva et al., [20] also found the highest population density of *G. brimblecombei* in the dry season of the year, in Minas Gerais.



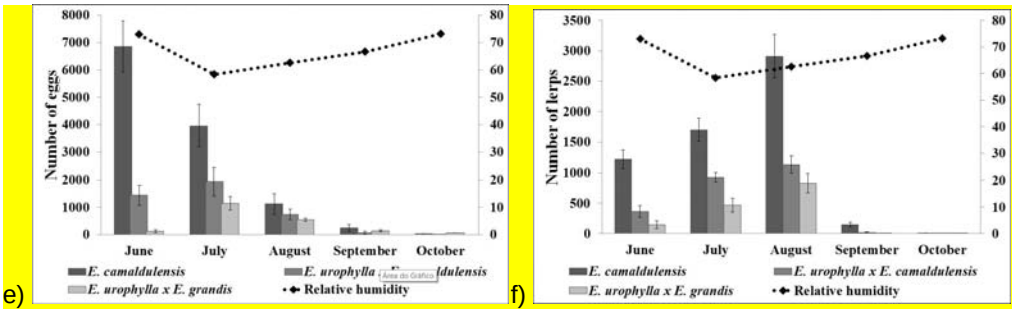


Figure 1 Population fluctuation of eggs (a, c, e) and lerps (b, d, f) of *Glycaspis brimblecombei* (Moore), in *Eucalyptus* with respective environmental factors in Juscimeira, MT (2016).

The infestation of experimental planting declined gradually after the month of August following the behavior of the rains. The region interferes in the pest infestation period, Montes y Raga, [21] studied the population dynamics in the Western São Paulo state and found that the greatest severity of attack occurred in the month of January. In a study on the biology of *Blastopsylla occidentalis* Taylor (Hemiptera: Aphalaridae), Soufo & Tamesse, [22] observed the numerical variation of abundance of psyllids in three periods of outbreaks during the year. The distinction of the psyllid-lerps and other psyllids is observed regarding their passivity concerning environmental factors of precipitation and humidity.

According to Oliveira et al., [23] the rain as a control agent of *G. brimblecombei* in seedlings of *E. camaldulensis* caused two synergistic effects on the insect. The first is the leaf wetness effect, which can lead to the solubility of the lerp sugars, exposing the nymphs to adverse environmental conditions or to predators attack, as well as the moisture that can cause indirect effects on nymphs due to the ease of occurrence of entomopathogenic fungi. The second effect is the mechanical removal of young individuals by the impact of water droplets, considering that the direct action of raindrops on the leaves of *E. camaldulensis* is also an important factor in population control of red gum lerp psyllid.

It was observed that *E. camaldulensis* always showed the largest quantities of eggs and lerps, due to the fact of favoritism by regional mean temperature. According to Firmino-Winckler, [4], the temperature of 26ºC is the most suitable for the nymphs growth of *G. brimblecombei*, observing that the viability of nymphs was 74%, while in the temperatures 18º and 22ºC the mean viability was 40.5%. In the hybrid "urograndis" the infestation occurred with a number of individuals always lower than the other eucalyptus.

The time of occurrence of this pest is associated to the pluviometric indexes [13], a result obtained by Silva et al., [20] who described the increase of the population inversely proportional to the relative humidity, with a higher number of individuals in the drought period. Soufo & Tamese, [22] also observed the influence of temperature on the number of individuals of *B. occidentalis*. The biological cycle of *G. brimblecombei* varies with the environmental conditions, however, through the Pearson's correlation test, it was not possible to prove the strong positive correlation between eggs and lerps (r = 0.8963), the possible prediction of the attack rate of red gum lerp psyllids to forest plantations with eucalyptus (Table 4).

Table 4. Pearson correlation for *Glycaspis brimblecombei* (Moore) in attack to *Eucalyptus* with respective environmental factors in Juscimeira, MT (2016).

Variables	Stage	*	Meteorological variables		
			Precipitation	Temperature	Moisture
E. camaldulensis	Eggs	r	-0.6326	-0.9543	0.0707
		t	2.4502*	9.5741**	0.2126^{ns}
	Lerps	r	-0.4328	-0.4362	-0.6033
		t	1.4403^{ns}	1.4543^{ns}	2.2692*
“urocam”	Eggs	r	-0.6821	-0.8517	-0.4665
		t	2.7979*	4.8750**	1.5821^{ns}
	Lerps	r	-0.4303	-0.4112	-0.7558
		t	1.4299^{ns}	1.3532^{ns}	3.4627*

"urograndis"	Eggs	r	-0.4103	-0.2704	-0.9177
		t	1.3498^{ns}	0.8425^{ns}	6.9278**
	Lerps	r	-0.2980	-0.2464	-0.6989
		t	0.9364 ^{ns}	0.7627 ^{ns}	2.9317*

*Data of the Pearson correlation; (ns) - not significant below 10%.

In the population fluctuation of collected insects there were only negative and reversed correlations, concerning the three environmental factors. The occurrence of rainfall leads to the fall of lerps of *G. brimblecombei* and the fungi growth on the leaves [23]. In *E. camaldulensis* the quantity of eggs showed correlation to rainfall and temperature, for lerps the correlation was only regarding humidity. That is such relevant information considering that rain has regulating effect on the populations of this psyllid in up to 96% of reduction [23].

In the hybrids only the amount of eggs sampled in 'urocam" had correlation with precipitation and temperature, since their quantities of lerps and the evaluated stages of "urograndis" were correlated to moisture. Sooker et al., [24] confirmed the relationship between the reduction of populations of *G. brimblecombei* with the arrival of periods of intense rainfall and that studies performed in Australia with *Glycaspis baileyi* indicated that the climate is an adjustment factor of the population of these insects and other species of psyllid.

The mineral formulations of fertilization, conventionally recommended, without considering the individual needs of each forest species cause a plant imbalance and make them attractive to the preference of the red gum lerp psyllid to the potential host.

CONCLUSIONS

In *E. camaldulensis* the omission of N and K are involved in preference and the absence of silicon causes the favoring of the pest oviposition. In the hybrid *E. urophylla* x *E. grandis* the formulations without addition of fertilization and N, P, K, are less preferred, perhaps due to the nutritional imbalance of conventional fertilization associated to the hybrid genetic resistance. In the hybrid *E. urophylla* x *E. camaldulensis* only genetic resistance had expression, due to the fact that the treatments had no significant effect to the pest preference.

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