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EFFECT OF ENDOZOOCHORY ON THE PHYSIOLOGICAL QUALITY OF JUAZEIRO, LEUCENA, TAMARIND AND UMBU SEEDS

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ABSTRACT

Seed dispersal has its importance for the biodiversity of our biomes, among the methods of seed dispersal is endozoochory, which occurs after the ingestion of fruits and excretion through the animal's feces. The objective of this study was to analyze the feasibility of overcoming dormancy in seeds of juazeiro, leucaena, tamarind and umbu after passing through the digestive tract of cattle, goats, sheep, donkeys and pigs, and to identify which of the forest species studied has the best germination viability and which animal is the most effective disperser after ingestion and excretion of these seeds. Seed water content, seedling emergence and vigor were evaluated through the variables, emergence speed index, length and dry matter of primary root and shoot of seedlings. The statistical design adopted was DIC, with the treatments in a 4 x 6 factorial scheme (plant species x animal species + control), the data were submitted to analysis of variance by the "F" test, at 5% probability and compared by Tukey's test. It was concluded that the dormancy of the researched seeds is overcome by the passage in the digestive tract of ruminants and monogastrics.

Keywords: Seed dispersal. Dormancy. Zoochory.

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INTRODUCTION

There are several agents that contribute to the seeds moving away from the mother plant, among them the abiotic dispersing agents by hydrochoric (dispersion by water), anemochoric (wind) and barochoric (gravity) mechanisms and the biotic ones evidencing zoochoric patterns such as birds, ants and small mammals, for example, which vary according to the characteristics of the seeds and fruits. (GONÇALVES et al., 2021).

Endozoochory is considered an ecological and evolutionary interaction, referring to the consumption of fruits or seeds by grazing animals and released through fecal matter. In this way, these seeds are dispersed throughout the field (Garcia, 2021). Endozoochory performed by animals can help with seed dispersal (Abbas et al., 2020).

The juazeiro tree, *Ziziphus joazeiro* Mart., belonging to the Rhamnaceae family, popularly known as juazeiro, (LORENZI, 2016), the species *Ziziphus joazeiro* has great economic, ecological and medicinal importance in the semi-arid region. Due to the presence of an extremely hard and resistant endocarp, the seedling emergence rate is very low, usually occurring between 70 and 100 days (LORENZI, 2016).

The leucaena, *Leucaena leucocephala* (Lam.) de Wit., belongs to the family Fabacea. Considered a very promising forage in the semi-arid region, leucaena has the ability to adapt to edaphoclimatic conditions in the Northeast region, showing a high capacity for regrowth, even in times of water scarcity (DIAS, et al., 2022).

The tamarind tree (*Tamarindus indica* L.) is a fruit tree of the Fabacea family and subfamily Ceasalpinoidea is originally from Africa, from where it has dispersed to several countries with tropical and subtropical climates. Brought to Brazil, tamarind seedlings have adapted very well in several states, especially in the Northeast due to the semi-arid climate (AZEVEDO et al., 2021). It is found throughout Brazil. It can reach 25 m in height, its fruits are elongated pods, 5 to 15 cm long and have a dark, woody and brittle brown skin, housing between 3 and 8 seeds surrounded by a brown and acidic pulp (FERNANDES et al., 2023).

The increase in the percentage of germination of these seeds can be promoted with treatments to overcome dormancy, such as soaking in water for 24 h; scarifying the seed coat; and scarifying + soaking in water for 24 h (PEREIRA et al., 2019).

The umbu *(Spondias tuberosa*) is a xerophytic anacardiaceous, small tree, which is 4 to 8 m tall and has an umbeliform crown. The trunk is short and wrapped in a smooth bark of 40 to 60 cm in diameter and the leaves are made up of membranous leaflets. The fruit is an ellipsoidal, glabrous or slightly hairy drupe, which has an epicarp

of variable thickness, greenish-yellow color (ROCHA, et al., 2024). Endemic to Brazil, this species has confirmed occurrence in the Northeast region (FLORA DO BRASIL, 2019).

The propagation of *Spondias tuberosa* can occur vegetatively, in the form of grafting, cuttings, micropropagation, and by seeds, which is very important for increasing the variability of the species, whose dispersion is zoochoric: deer, caputtus, goats and sheep are its main dispersers (OLIVEIRA et al., 2018). In a natural environment, germination occurs when seeds pass through the digestive system of ruminants (OLIVEIRA et al., 2018).

Large seeds are more prone to damage from chewing and digestive fluids, while smaller seeds cross the digestive tract with greater speed, thus decreasing their period of exposure to potentially stressful conditions of the digestive tract (PETERSEN & BRUNN, 2019).

The objective of this study was to evaluate the feasibility of overcoming dormancy in seeds of juazeiro (*Ziziphus joazeiro* Mart.), leucaena (*Leucaena leucocephala* (Lam) de Wit.), tamarind (*Tamarindus indica* L.) and umbu (*Spondias tuberosa* Arruda) after passing through the digestive tract of ruminants: sheep (*Ovis aries*), goats (*Capra aegagrushicus*), cattle (*Bostaurus indicus*), and of the monogastrics: donkeys (*Equus asinos*), and swine (*Suis scrofadomesticus*). To identify which of the studied forest species has greater germination viability and which animal is the most effective disperser after the ingestion and excretion of these seeds, thus providing the balance of the rational exploitation of the forest resources of the Caatinga with agriculture, increased by the increase in plant and animal production.

MATERIAL AND METHODS

PLACE AND PERIOD OF THE STUDY

The feeding of the ruminant animals, *Ovis aries*, *Capra aegagrushicus* and *Bostaurus indicus* and the monogastric animals *Equus asinos* and *Suis scrofadomesticus*, were conducted in collective stalls by species, belonging to the Federal Institute of Education, Science and Technology of Paraíba - IFPB, Campus Sousa, PB. The evaluation of the emergence and vigor of the seeds of the fruits of *Ziziphus joazeiro*, *Leucaena leucophala*, *Tamarindus indica* and *Spondias tuberosa*, after passing through the digestive tract of the animals was carried out in the greenhouse and later in the Seed Analysis Laboratory - LAS, of the Department of Plant Science and Environmental Sciences of the Federal University of Paraíba - UFPB, Campus II, CCA, Areia, PB. The harvests of the fruits took place in the 2018 and 2019 harvests.

FRUIT OFFERINGS TO ANIMALS

Table 1. Plant species studied with the quantity and weight of seeds, offered to animals by species

Plant species	Amount of fruits/pod	Seed quantity	Average weight (g)
Ziziphus joazeiro	200	200	1.060*
Leucaena leucocephala	115	2.530	200**
Tamarindus indica	200	914	1.960***
Spondias tuberosa	200	200	2.845*
*:fruit (peel, hoopoe and endocarp); **: pod with seeds; : fruit (hoopoe and seed without the shell).			

The fruits were added to the energy concentrate of ground corn, in the proportion of 3% of the animal live weight and there were no restrictions regarding the amount of water ingested.

VARIABLES ANALYZED

SEED WATER CONTENT

The water content of the seeds/endocarps of *Ziziphus joazeiro*, *Leucaena leucocephala*, *Tamarindus indica* and *Spondias tuberosa*, were determined according to the methodology prescribed in the Rules for Seed Analysis (RAS) (BRASIL, 2009), with modifications, using the greenhouse method, at 105 ± 3°C, for 24 hours, with two replications of 10 seeds of each species, and the results were expressed as a percentage with base on the wet weight of the seeds/endocarp.

EMERGENCY PERCENTAGE

The tests to evaluate the seedling emergence of *Ziziphus joazeiro*, *Leucaena leucocephala*, *Tamarindus indica* and *Spondias tuberosa*, were installed jointly in a greenhouse of the LAS (with an average temperature of 34°C and relative humidity of 70%), in four replications of 25 seeds per treatment, totaling 100 seeds sown in polyethylene trays, containing as substrate, sand washed and sterilized in an autoclave at 120°C for 120 minutes, whose humidity was maintained by means of daily watering with manual watering can. The count of the number of normal seedlings emerged was performed daily, considering the emission of the shoot with the presence of roots until the cotyledon leaves were fully open. The results were expressed as percentages.

EMERGENCY SPEED INDEX

The Emergence Speed Index (IVE) determined from the first seedlings emerged until their stabilization, divided by the number of days elapsed between sowing and emergence.

PRIMARY ROOT AND SHOOT LENGTH OF SEEDLINGS

The length of the normal seedlings of *Ziziphus joazeiro*, *Leucaena leucocephala*, *Tamarindus indica* and *Spondias tuberosa*, was determined at the end of the emergence test, for this, the primary root and the aerial part of each seedling were measured, individually, with the aid of a ruler graduated in centimeters and the results had their values expressed in cm seedling.

ROOT AND SHOOT DRY MATTER OF SEEDLINGS

Soon after the length measurements were inferred, the normal seedlings of each replication were divided into roots and shoots, packed in Kraft paper bags and kept in an oven with forced air circulation regulated at 65°C until constant weight was obtained, approximately 48 hours, then the samples were weighed on an analytical scale with a precision of 0.001g and the result expressed in g seedling.

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The application of statistical techniques to analyze or synthesize the study data was the Entirely Randomized Design (DIC), with the treatments distributed in a 4 x 6 factorial scheme (plant species x animal species + control), with four replications of 25 seeds. The data were transformed into an arc sine x 100 to normalize the distribution of deviations that occur when seed germination is analyzed (PIMENTEL-GOMES, 2022) and subsequently submitted to the Shapiro-Wilk normality test (SHAPIRO and WILK, 1965). Once the assumptions of normality were met, the data were submitted to analysis of variance (ANOVA) by the F test, at 1% probability of error. The means of the variables significantly influenced by qualitative factors (plant and animal species) were compared by the test of multiple comparisons of means (Tukey's test, at 5% probability). The analysis was processed using the Sisvar software, version 5.6 (FERREIRA, 2014b).

RESULTS AND DISCUSSION

The interaction between the factors: Emergence Percentage (EP), Emergence Speed Index (IVE), Primary Root Length (CR), Shoot Length (CPA), root dry matter and shoot dry matter for the animal species, *Ovis aries*, *Capra aegagrushicus*, *Equusasinos* and *Suis scrofadomesticus* and *Bostaurus Indicus*, and plant species, *Ziziphus joazeiro*, *Leucaena leucocephala*, *Tamarindus indica* and *Spondias tuberosa*, and their interactions of animal species and plant species was significant at 1% probability, in the harvests of these species studied in the years 2018 and 2019 by the F test, for all these variables

analyzed, except for the primary root length of seedlings, from seeds harvested in the harvest 2019. These results indicate that the viability and vigor of seeds excreted in animal feces vary depending on the characteristics of the plant species and the animal species that ingested the fruits and/or seeds.

Table 2. Analysis of variance for percentage of emergence, emergence speed index, primary and shoot root length, and root and shoot dry matter of seedlings of *Ziziphus joazeiro*, *Leucaena leucocephala*, *Tamarindus indica* and *Spondias tuberosa*, from seeds after passing through the digestive tract of ruminants (sheep, goats and cattle) and monogastric (donkeys and pigs)

Sources of variation	GL	Medium Squares (2018 Vintage)					
		PE	IVE	CR	CPA	MSR	MSPA
Plant species (V)	3	563.99**	153,56**	250,42**	299.67**	42,74**	132,24**
Animal species (A)	5	199.95**	15,24**	83.11**	117,36**	20,63**	12,55**
V x A	15	1367.68**	45,28**	206,00**	109,61**	45,42**	21,23**
Error	72	45,91	4,46	8,18	6,87	2,34	2,61
CV (%)		25,09	36,49	17,16	16,65	29,16	40,90
		Medium Squares (2019 Vintage)					
Plant species (V)	3	2477.49**	303.47**	2091.10**	1167.46**	181,96**	243.69**
Animal species (A)	5	1620,50**	98.10**	58.81ns	67,79**	16,77**	36.99**
V x A	15	435.22**	53,74**	89,94**	96.40**	12,66**	28,22**
Error	72	125,32	10,98	35,75	15,37	3,41	3,79
CV (%)		40,40	44,87	46,17	40,53	64,00	62,70

ns, * and **: not significant, significant at 1 and 5% probability, respectively, by the F test; GL: degree of freedom; PE: emergency percentage; IVE: emergency speed index; CR: primary root length; CPA: length of aerial part; MSR: root dry matter; MSPA: shoot dry matter; CV: coefficient of variation. The data transformed into "arc sine " √("X/100").

The water content of the seeds/endocarps of *Ziziphus joazeiro*, *Leucaena leucocephala*, *Tamarindus indica* and *Spondias tuberosa* did not show significant differences, none of the treatments stood out among them. All seeds, after passing through the digestive tract of the animals, were sanitized and selected, under the same conditions: location, time and temperatures. The seeds studied are orthodox, they undergo a drying process during their maturation inside the fruit and later an artificial drying process when released by the mother plant, and in the conditions studied an environment full of microorganisms and high humidity, as is the case of the digestive tract of animals, it can accelerate the germination process.

When we refer to the Percentage of Emergence (EP), the seeds that passed through the intestinal tract of *Ovis aries*, the highest seedling PE occurred in *the species Leucaena leucocephala*, with 67%, this result of germination in sheep, in 2018, was the highest index among animal species and among all plants studied. Results above 50% of germination beyond this were verified only in the seeds of the *Tamarindus plant indicates* that it showed a higher percentage of emergence in the control treatment (they did not pass through the

digestive tract of the animals), (86%) in 2018 and with the control of *Ziziphus joazeiro* (78%), in 2019.

The values of percentage of seedlings emerged below 50% suggest possible natural mechanisms of dormancy of the seeds of *Leucaena leucocephala*, *Tamarindus indica* and *Spondias tuberosa, whose action of the seeds of Leucaena leucocephala, Tamarindus indica and Spondias tuberosa, whose action of the seeds of the seeds of Leucaena leucocephala, Tamarindus indica and Spondias tuberosa* may have caused the low percentage of seedlings emerged, notably in combinations of environmental conditions that would be favorable to germination.

In 2018 and 2019, the seeds of *Tamarindus indicate* that they were not offered to the animals (control) showed the highest percentage of seedling emergence (86% and 37%), respectively, with a statistically significant difference in relation to the seeds that were ingested, both by ruminants and monogastrics, except for Capra *aegagrushicus* in 2019, which did not differ statistically.

Azevedo, et al. (2023); Abbas et al., (2020). When studying the seed dispersal by enzoochory in cattle, they observed that this process did not affect the viability of the ingested seeds and that these animals are able to supply the seed bank in the pastures, and the mechanical milling during chewing and rumination by ungulates, as well as the retention in the intestine and the exposure to digestive juices, can affect seed survival and germination, respectively.

Table 3. Emergence of seedlings of *Ziziphus joazeiro*, *Leucaenal eucocephala*, *Tamarindus indica* and *Spondias tuberosa* from seeds excreted in the feces of ruminants and monogastrics and removed from the fruits (control) in the years 2018 and 2019

Animals	*Ziziphus joazeiro*	*Leucaena leucocephala*	*T. indica*	*Spondias tuberosa*
Year 2018				
Witness	17bAB	8bC	86aA	7bBC
Sheep	32bA	67aA	0cD	21bAB
Goat	26abA	36aB	16bCD	16bAB
Asinine	27aA	43aB	41aB	2bC
Swine	30aA	5cC	10bcCD	26abA
Cattle	0bB	28aA	28aBC	18aAB
Year 2019				
Witness	78aA	43bA	37bA	39bA
Sheep	21abBC	39aA	9bB	34aA
Goat	43aB	33abA	10bAB	34abA
Asinine	28aBC	14aA	8aB	29aA
Swine	44aB	23aA	2bB	0bB
Cattle	15bC	41aA	1bB	12abAB

Means followed by the same letter, lowercase in the row and uppercase in the column, do not differ between the Scott-Knott clustering test, at 5% probability.

The Emergence Speed Index (IVE), the results suggest that the plant species *Leucaena leucocephala,* in the years 2018 and 2019, has greater sensitivity to the processes occurring in the digestive tract of *Ovis aries*, *Capra aegagrushicus*, and *Bostaurus Indicus*, *Equusasinos* and *Suis scrofadomesticus*, since the emergence of seedlings of this species occurred in a shorter time. Mariano et al. (2016) comment that, in case of persistent dormancy, other methods of overcoming numbness are recommended, such as mechanical scarification with 100 grit sandpaper; immersion in sulfuric acid (H2SO4) (95%) for 4 minutes and immersion in water (H2O) at 80°C for 15 minutes.

Table 4. Seedling emergence speed index of *Ziziphus joazeiro*, *Leucaena leucocephala*, *Tamarindus indica* and *Spondias tuberosa seedlings* from seeds excreted in ruminant and monogastric feces and removed from fruits (control) in 2018 and 2019

Animals	*Ziziphus joazeiro*	*Leucaena leucocephala*	*T. indica*	*Spondias tuberosa*
Year 2018				
Witness	0.68bAB	0.68bC	4.92aA	0.35bA
Sheep	0.95abA	2.32aB	0.00cD	0.56bA
Goat	0.84abA	2.41aB	0.61bCD	0.41bA
Asinine	0.58cAB	6.45aA	2.65bAB	0.26cA
Swine	1.33abA	1.39abBC	2.50aAB	0.42bA
Cattle	0.00cB	3.06aAB	1.59abBC	0.84bA
Year 2019				
Witness	2.82aA	3.83aAB	1.20aA	1.88aB
Sheep	0.77bA	7.19aA	1.64bA	8.15aA
Goat	1.87bA	4.17abAB	1.49bA	8.15aA
Asinine	0.56aA	2.76aB	0.88aA	0.42aBC
Swine	1.53abA	3.94aAB	0.50bcA	0.00cC
Cattle	0.73bA	6.16aAB	0.25bA	0.65bBC

Means followed by the same letter, lowercase in the row and uppercase in the column, do not differ between the Scott-Knott clustering test, at 5% probability.

The Ovis aries, *Capra aegagrushicus* feeding on *Leucaena leucocephala seeds and* Spondias tuberosa *endocarps* obtained the highest percentage of IVE, 7.19 and 8.15% respectively, comparing them with *Bostaurus Indicus, Equus asinos* and *Suis scrofadomesticus*, even higher rates than the control group. The seedling emergence speed index of *Ziziphus joazeiro* and *Tamarindus indica species* did not differ as a function of the animal species evaluated.

In *Leucaena leucocephala*, the highest emergence speed was obtained when seedlings were originated from seeds fed to *Ovis Aries* (7.19), but without statistical difference from those seeds offered to *Capra aegagrushicus* (4.17), *Suisscro fadomesticus* (3.94), *Bostaurus indicus* (6.16) and also from the control (3.83). The reduction in the emergence rate index of *Tamarindus seedlings indicates* that the seeds passed through the digestive tract of *Ovis aries*, *Capra aegagrushicus* and *Bostaurus indicus* may have occurred due to possible damage to the seeds, the occurrence of cracks by the chewing

apparatus or prolongation of the exposure time. The seeds of *Spondias tuberosa*, in the animal and control species used in 2018 did not promote statistical variations.

The studies of the Primary Root Length (CR) showed that *Leucaena leucocephala* presented the highest CR, 22.5 cm among the other plants researched and *Ziziphus joazeirose stood out with all animals in at least one year of the research. On the other hand, Tamarindus indica* and *Spondias tuberosa* obtained better averages in three of the five species of animals studied, distributed as follows: *Capra aegagrushicus*, *Suisscro fadomesticus*, *Bostaurus Indicus* for the first and *Ovis aries*, *Capra aegagrushicus* and *Bostaurus Indicus* for the second.

Analyzing the Shoot Length (APC), it was observed that *Spondias tuberosa* presented better results in the two years researched compared to *Ziziphus joazeiro*, *Leucaena leucocephala* and *Tamarindus indica*, including for the control group, obtaining a longer shoot length, 14.11 cm. Also all the animals studied, sheep, goats, donkeys, pigs and cattle, when obtained the seeds of *Spondias tuberosa* and *Tamarindus indica* in the feces showed higher averages for this variable. Studies of genetic divergence are characterized by important parameters to determine how genetically distant a population or genotype is from another, in this sense, the variability in the length of the area part between the plant species studied may be related to genotypic and phenotypic divergence (CRUZ, 2018).

The increase in primary root length may be related to the greater availability of nutrients resulting from microbiological interactions in the substrate, notably because root growth is minimal in poor soils, as they become limited by nutrients. As the availability of nutrients in the soil increases, roots proliferate, while soil nutrients exceed an optimal concentration, root growth can become limited by carbohydrates. In some species, with high concentrations of nutrients in the soil, a few roots are sufficient to supply all the necessary nutrients, so the plant can decrease the allocation of its resources to the roots while increasing its allocation to the shoot and reproductive structures (TAIZ, et al, 2017).

The amount of seed reserves is directly associated with the functional morphology of cotyledons, which may have significantly affected the germination and development of these species (DRANSKI, et al, 2019). These researchers report that the initial growth of plants depends not only on the inherent performance potential of the seed, but also on the environmental and edaphic conditions of treatment and cultivation, such as the passage of seeds through the digestive tract of these animals, which may have influenced the expression of physiological potential.

Between Shoot Dry Matter (MSPA) and Root Dry Matter (SRM) treatments, the accumulation of these is a result of the growth process, which depends on the initial

phases, such as germination and emergence, therefore, the growth differences between plant species reflect in the percentage and speed index of seedling emergence. Thus, the seedlings that accumulated greater root phytomass may have benefited from the fact that they emerged in less time and, therefore, formed the light capture system more quickly, which favored greater photosynthetic activity and phloem translocation of photosynthates for nutrition of growing roots (TAIZ, et al, 2017).

In the seeds that were not offered to the animal species, the highest root dry matter content was obtained in seedlings of *Tamarindus indica* (6.52 g), whose value differed statistically in relation to the species *Spondias tuberosa* (1.03 g), *Ziziphus joazeiro* (0.27 g) and *Leucaena leucocephala* (0.11 g) and whose value was statistically higher than those obtained from seedlings originated by seeds that passed through the digestive tract of ruminants and monogastrics.

In 2019, we verified that the dry matter values of *Leucaena leucocephala* seedling roots did not differ statistically between the treatments, that is, the passage of the seeds of this species through the digestive tract of the ruminants and monogastrics used have no influence on their root dry matter content.

In 2018, from the unfolding of the effect of animal species within each plant species, the ruminants and monogastrics used did not provide effects responsible for the differences in the accumulation of dry matter of aerial part of seedlings of *Ziziphus joazeiro*.

The passage of seeds through the digestive tract of the animals, under the conditions in which they were studied, was efficient in overcoming dormancy, given that, after this practice, higher emergence values were recorded for the year 2018 and the same for the year and 2019 compared to the control group, except for *Ziziphus joazeiro* in 2018, for the *Bostaurus Indicus*.

In 2019, from the unfolding of animal species within the plant species, it was found that the seedlings of *Ziziphus joazeiro* and *Leucaena leucocephala* did not express a significant difference in shoot dry matter values between the treatments (ingested seeds and seeds not offered to the animals).

Possibly the germination process and seedling emergence were influenced by the anticipation of seeds coming from the passage through the digestive tract of animals where they have microorganisms that interact with the substrate where they were sown, which, in turn, consists of an organic input rich in nutrients and beneficial microorganisms, especially nitrogen and potassium (CHOJNACKA, et al, 2020). And it can respond to the changes that have occurred in the growth profiles of plant species seedlings, as the use of biological

inducers as microorganisms can be a promising alternative for promoting plant growth (EL-MAGEED, et al, 2020).

CONCLUSION

It was found that the dormancy of the seeds of *Ziziphus joazeiro*, *Leucaena leucocephala*, *Tamarindus indica* and *Spondias tuberosa* is overcome by the passage in the digestive tract of ruminant (*Bostaurus indicus, Ovis aries*, *Capra aegagrushicus*) and monogastric (*Equus asinos* and *Suisscrofa domesticus*) domestic herbivores.

In the variables analyzed for plants and animals, it was observed that *Ziziphus joazeiro*, *Leucaena leucocephala* and *Spondias tuberosa* were similar to each other and superior to *Tamarindus indica*.

No superiority was found in terms of plant dispersion among animals, in terms of the percentage of seedlings emerged and emergence speed indexes.

There was no superiority in terms of dormancy breakdown for all the variables studied among monogastric patients: *Suis scrofadomesticus; Equus asses* and ruminants and: *Ovis aries*; *Capra aegagrushicus; Bostaurus Indicus Indicus Indicus,* nor of one animal species over another.

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