

Factors that influence in *Jatropha curcas* L. latex production

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ABSTRACT: Latex from *Jatropha curcas* L. exhibits biotechnological potential for the development of biodiesel and drugs. Little is known about the collection methods and latex productivity of this species. In order to garner information on the use of *J. curcas* latex, factors that influence production by this species were assessed. As a result, no significant difference was found between the volume of latex collected in the stem and the branches. With respect to environmental characteristics, climatic conditions, such as low temperatures and rainfall, affected production. The period of low temperatures and leaf fall occurs after fruit harvest and, in the absence of strong sinks, the plant can use the assimilates to generate greater latex

production. Multiple regression analysis demonstrated that latex production is positively associated with plant height and negatively associated with fruit production and other variables that maximize it. During the fruiting process the plant uses most of the assimilates for fruit development and a lower percentage to produce secondary compounds, resulting in less availability of these metabolites for latex production. In general, the latex production obtained from this species is low and limits commercial exploitation of this compound, despite its possible pharmacological potential.

Key words: Physic nut, biodiesel, pharmacological potential, environmental conditions.

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INTRODUCTION

Jatropha curcas L., popularly known as physic nut, is a monoecious, perennial lactiferous species belonging to the family Euphorbiaceae (Laviola et al. 2015). In recent decades it has attracted considerable attention due to its high economic potential. The plant has multiple uses in different parts of the world. The oil from the seeds is used in cooking, as lighting fuel, as biopesticide and, in popular medicine, to treat various ailments (Contran et al. 2013). The plant can be used as a hedge or source of organic matter; however, since it is an oleaginous species, the main economic interest is exploiting the high seed oil content ($\pm 35\%$) for biodiesel production (Dias et al. 2007; Matos et al. 2014a). The physic nut, a rustic plant with high edaphoclimatic adaptability, develops under marginal soil fertility, salinity and drought conditions (Dias et al. 2007; Matos et al. 2014b). These characteristics have prompted the expanding cultivation of the physic nut in Brazil, especially in regions unsuited to drought-sensitive crops. It is estimated that production aimed at oil extraction encompasses over 30 thousand hectares in Brazil (Ferreira et al. 2013). Despite the high economic potential of the physic nut, there are significant concerns over cultivating the species:

- i. There is little information on the basic agronomic aspects that ensure profitable yields;
- ii. It is a wild species without genetic improvement (Maes et al. 2009); and
- iii. The pharmacological and physiological applicability of latex production remain largely unknown.

Numerous studies are underway to elucidate the morphological, biochemical and agronomic aspects in order to maximize the potential of this species (Laviola et al., 2015; Bhering et al. 2013; Veronesi et al. 2012; Behera et al. 2010), such as using latex as product with medicinal applications (Katagi et al. 2016; Costa et al. 2014; Thomas et al. 2008).

Latex obtained from the physic nut has been used in traditional medicine to treat a number of conditions, such as burns, hemorrhoids, mycoses and ulcers (Debnath and Bisen 2008). In addition to popular use, studies have shown that physic nut latex exhibits anticoagulant activity (Osonlyi and Anajobi 2003). Moreover, phytochemical analyses demonstrate that the latex of this species contains natural components with cytotoxic potential, including

curcacyclins A and B (Insanu et al. 2012) and curcusesomes A, B, C and D (Aiyelaagbe et al. 2011); anticancerous compounds, such as the protein curcin (Jaramillo-Quintero et al. 2015) and the alkaloids jatrophine and jatropham (Thomas et al. 2008); and antibactericidal and antimalarial compounds, such as curcacyclins A and B and jatrophidin (Sabandar et al. 2013). Thus, extraction of these latex biocompounds would add commercial value to this cultivar, which could be used to produce biodiesel and drugs.

Although *J. curcas* L. belongs to the same family as the rubber tree, the most widely used plant to obtain latex in the tire industry, there is no information in the literature on physic nut latex production. Thus, the present study aims to assess the influence of the following variables on physic nut latex production:

- i. Collection method;
- ii. Space between plants;
- iii. Environmental conditions, such as temperature, humidity and rainfall;
- iv. Phenological characteristics (presence of flowers and fruits);
- v. Morphological characteristics, such as height, crown size and stem diameter; and
- vi. Physiological characteristics, such as relative water content and total chlorophylls.

MATERIAL AND METHODS

Experimental area and climatic data

Latex samples were collected from four-year-old plants, cultivated in field conditions at Goiás State University (UEG), in the municipality of Ipameri (lat 17° 43'19"S, long. 48° 09'35"W, alt. 773 m). The climate is classified as Aw (tropical seasonal) with annual rainfall of about 1,600 mm and is characterized by two distinct seasons, dry from April to October and rainy from November to March, with temperatures averaging around 23 °C (Alvares et al. 2014). The soil of the experimental area is classified as red-yellow latosol. Liming and fertilization were performed based on soil analysis and following technical recommendations (Matos et al. 2014a, Table 1). The experiment was conducted using a completely random design and plants grown in three spacings (3 × 3, 3 × 2 and 3 × 1 m), with six repetitions and one plant in each experimental plot.

Table 1. Soil chemical characteristics, from 0 to 20 cm depth and 20 to 40 cm, of the experimental area of the UEG - Ipameri - GO Campus.

Depth	pH in CaCl ₂	Organical material (g·dm ⁻³)	P-Mehlich (mg·dm ⁻³)	Components (cmolc·dm ⁻³)					Base saturation (%)	
				K	Ca	Mg	Al	H+Al		Cation Exchange Capacity
0 to 20	5.6	33.0	7.0	0.20	3.4	1.3	0.0	3.5	8.4	58.3
20 to 40	5.3	37.0	8.8	0.25	4.2	1.6	0.0	3.7	9.8	62.0

Selection of study material

The height of the crown and stem and branch diameter of all the physic nut plants in the experimental field were measured using a tape measure and digital caliper in order to select homogeneous plants. The experiment lasted 10 months (October/2014 to July/2015) and data were collected on the 20th of each month, starting at 6 a.m.

Bleeding method and latex production:

Latex productivity was measured by collecting this material from the selected trees, using two collection methods (F and R). The F method is characterized by a vertical 10-cm cut in the stem made with a knife. For the R method, a 3 to 5-cm vertical cut is made in the branch, also with a knife. Both cuts were 0.5 cm deep. The latex was stored in 50 mL Falcon tubes to which no anticoagulants were added.

The productivity of each plant was determined according to the volume of latex obtained from bleeding in the different experiments. Bleedings were performed once a month for ten months, on a rainless day, starting at 6 a.m. and ending at around 8 a.m. Temperature, humidity and rainfall were measured monthly.

Physiological variables and fruit production

Plant height, as well as stem and crown diameter, were measured using a digital caliper and tape measure. To determine leaf chlorophyll concentrations and total carotenoids, leaf discs from totally expanded leaves were collected from a known area and placed in jars containing dimethyl sulfoxide (DMSO). Next, extraction was carried out in a water bath at 65 °C for three hours. Aliquots were removed for spectrophotometric reading at 480, 646 and 665 nm. Chlorophyll *a* (Cl *a*) and *b* (Cl *b*) content were determined in accordance with the equation proposed by Wellburn (1994). The fruits were collected as soon as they

turned yellow, dried and weighed, with a moisture content of around 13%. The nonuniformity of fruit ripening extended the harvest from December to March.

To obtain relative water content, five 12 mm-wide leaf discs were removed, weighed and placed in Petri dishes containing distilled water for 24 h for saturation. Next, the discs were weighed again and dried at a temperature of 70 °C for 72 h to obtain dry matter weight, which was calculated using the following formula:

$$\text{Relative Water Content (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 10$$

Statistical Analysis

Data related to climatic factors were submitted to principal component analysis (PCA) by covariance, using the broken-stick criterion to designate the number of axes used for ordination. Variables with eigenvalues higher than 0.6 were included in the PCA graph. Permutational multivariate analysis of variance – PERMANOVA (Anderson 2001) – was used to compare whether there was a difference between the climatic variables of the two groups formed in ordination.

The data were standardized for the analyses (PCA and PERMANOVA), since they were expressed in different measuring units and Euclidian distances were used as measures of sample dissimilarity. Bleeding methods were compared using the Mann-Whitney U test and extraction times with the Kruskal-Wallis and Dunn tests. Moreover, in order to identify plant variables that contributed most to *J. curcas* L. latex production, multiple regression analysis was used to select the best model for the stepwise procedure, applying the information criterion proposed by Akaike (Hair et al. 2009). Statistical analyses were performed in the R program version 3.2 (R Development Core Team 2015).

RESULTS AND DISCUSSION

Assessment of latex production associated with bleeding method and plant spacing

Mean latex/plant/bleeding production over 10 months using the F and R methods were 0.93 mL and 1.07 mL, respectively. A comparison of the samples obtained in latex production between the different collection methods using the Mann-Whitney test showed no statistical difference in latex productivity between the bleeding methods used ($U = 3739.5$; $Z = -0.89$; $p = 0.37$). With respect to latex production associated with plant spacing, it was found that 3×3 spacing produced an average of 1.03 mL, 3×2 spacing 1.12 mL and 3×1 spacing 0.86 mL of latex during the 10-month collection period. The Kruskal-Wallis test was used to determine the effect of density on latex production. The test showed that spacing is not linked to *J. curcas* L. latex yield (significance value of the test), with similar data and non-significant data obtained in all the treatments ($H_{(2, n = 180)} = 2.52$; $p = 0.28$). However, when it was considered the latex productivity per hectare, the three spacing showed different production: 1.4, 1.86 and 2.86 L/ha for the 3×3 , 3×2 and 3×1 spacing, respectively ($H_{(2, n = 180)} = 30.66$; $p < 0.01$). Although the 3×1 spacing produces more latex, once it has more plants per hectare, it is not recommended to plant the physic

nut at 3×1 spacing because of the high competition for soil resources and low fruit yield (Figure 1; Cassiano et al. 2013). In addition, independently of the spacing used, the *J. curcas* latex productivity is very small when compared with other lactiferous, such as *Hevea brasiliensis* (higher than 1,000kg/ha/ano; Silva et al. 2010).

Assessment of latex production associated with environmental conditions and phenological characteristics

Variations in minimum temperature, rainfall and fruit yield explained 58% of the variations in latex production (Table 2). Principal component analysis and PERMANOVA made it possible to separate the samples into two significantly different groups (Pseudo F = 7.40; $p = 0.01$ - Figure 1). Group 1 is represented by samples obtained between October and April, characterized by elevated temperatures, high water availability, the presence of inflorescences and/or high fruit yield, green and exuberant foliage and low latex production. Group 2 is represented by samples obtained from May to July, characterized by low temperatures, scarce rainfall, absence of inflorescences, fruits and leaves, and high latex production (Figure 1). The results indicate that low temperatures, rainfall and fruit production are primarily responsible for physic nut latex production.

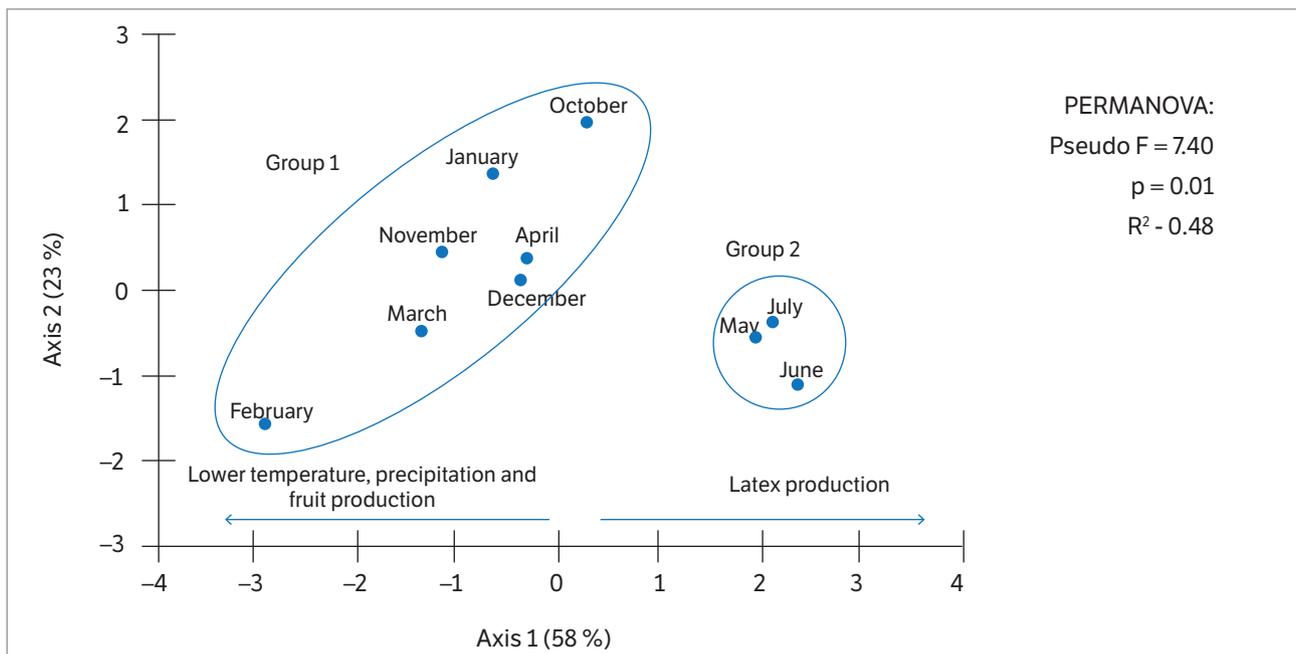


Figure 1. Ordination of principal component analysis scores (PCA) for climate data. The PERMANOVA was used to compare the two groups formed in ordination.

Assessment of latex production over the course of the year

Figure 2 illustrates yield data in different months of the year. The results indicate that October, November and December (flowering and fertilization period) exhibit reasonable latex production and absence of statistical difference. In January, February and March (fruit maturation and ripening), latex production is lower and statistically different from all the other months of the year. Latex production is highest in May, June and July, when plants are without inflorescences and leaves. The results indicate a close relationship between latex production and the morphological and productive characteristics of physic nut plants.

Assessment of latex production associated with physiological and productive variables

The multiple regression model, fit to the physiological and productive variables, was significant ($F_{(3, 176)} = 29.35$; $p < 0.01$; adjusted $R^2 = 0.32$) showing that plant height is positively related to latex production, whereas crown diameter and fruit yield had a negative effect on latex

production (Table 3). Variance partition (Figure 3) shows that fruits and the crown had the greatest influence on latex production, with individual contributions of 11% and 10%, respectively, and shared fraction of 10%. Taken together, these two variables explained 32% of variation in latex production. Height, in turn, exerted little influence

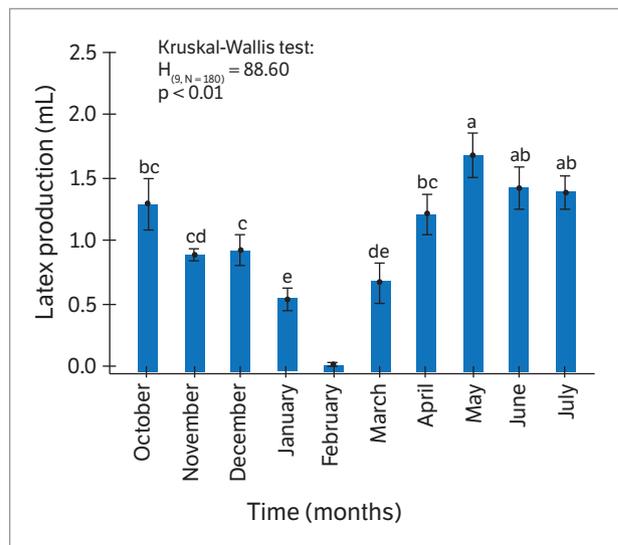


Figure 2. Comparison of average production of latex made over 10 months, using the Kruskal-Wallis test. Means followed by the same letter do not differ by Kruskal-Wallis test ($p > 0.05$).

Table 2. Meteorological data obtained in the collection months, latex yield in milliliters and number of fruits produced per month.

Month	Temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Humidity (%)	Precipitation	Latex (ml)	Fruits
October	26.5	30.9	19.2	80	103	23.3	-
November	27.0	29.4	19.7	83	143	15.9	-
December	26.0	29.5	20.1	90	276	16.6	-
January	26.0	28.5	19.9	85	377	9.6	-
February	25.0	31.3	19.9	87	267	0.0	3,251
March	23.0	29.5	19.6	88	188	12.1	1,213
April	22.5	29.5	18	84	165	21.9	489
May	21.5	28.3	15	81	93	30.2	204
June	20.0	28.4	13.2	82	37	25.5	-
July	19.0	28.2	13.2	78	4	25.0	-

Table 3. Multiple regression analysis to evaluate the influence of climate and phenological variables in *J. curcas* L. latex production.

Variables	Beta	Standard Deviation from Beta	t value	P
Intercepto	1.3965621	0.3143246	4.443	< 0.01
Fruits	-0.0026198	0.0004736	-5.532	< 0.01
High	0.4601745	0.0955994	4.814	< 0.01
Crown	-0.7600980	0.1442738	-5.268	< 0.01

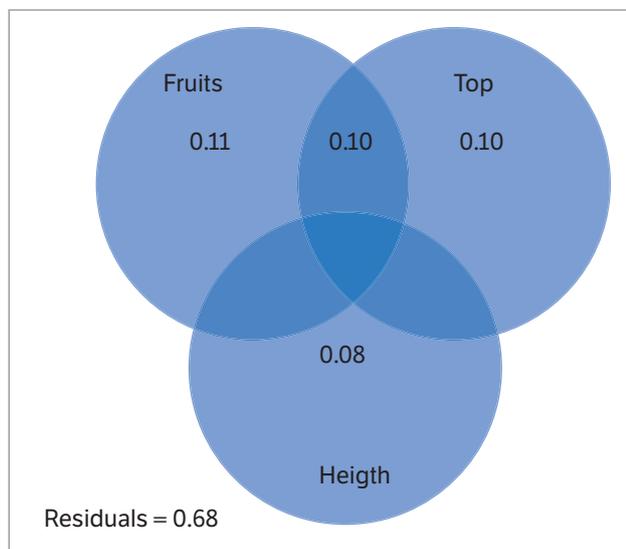


Figure 3. Diagram illustrating contribution of individual and shared variables that influence significantly ($p < 0.05$) *J. curcas* L. latex production.

on latex production, explaining only 0.8% of data variation (Figure 3). Relative water content (RWC), leaf chlorophyll concentrations (a + b) and total carotenoids assessed monthly exhibited no significant variations or relationships with latex production.

J. curcas L. is still considered a semi-wild species, or in the process of being domesticated. Its basic agronomic properties remain poorly understood and the effects of environmental changes on the species are being investigated (Contran et al. 2013). Specifically in terms of latex production, there is no information in the literature on collection methods, environmental factors that influence production or the commercial applications of latex, except its use in popular medicine. Thus, the results obtained in the present study are unprecedented and show certain particularities of this species in the production of this compound.

In relation to collection methods, statistical analysis showed no significant difference in the volume of latex collected in the stem and branches. It was also observed that spacing between the cultivars did not significantly interfere in latex production. However, other studies have shown that spacing affects other characteristics important for cultivation, such as the formation of clusters, number of fruits per cluster, production per plant and overall physic nut yield, where a higher yield was observed in treatments with lower plant density, that is, greater spacing (Müller et al. 2015).

Latex production depends on, among other things, the availability of carbohydrate reserves, nutritional status of the plant, anatomic structure of lactiferous vessels and biosynthesis of aminoacids and proteins (Melo et al. 2004). In addition to these factors, seasonal climate changes involving fluctuations in light, temperature and humidity can affect photosynthetic activity and, consequently, plant metabolism, altering latex production (Melo et al. 2004). For this reason, environmental data in the present study were obtained in the collection months, followed by assessment of the effect of these variables on latex production.

In regard to the environmental variables associated with physic nut cultivation, literature data show that climatic conditions can interfere directly with latex production, as well as with flowering and uneven fruit maturation (Brasileiro et al. 2012). Analyses of the relative water content (RWC) at the leaves were performed during each latex collection. Ours data showed that *J. curcas* is not suffering from water stress, once the medium value of RWC was 82.4%. The absence of a statistical difference in relative water content in *J. curcas* L. leaves demonstrates that the plant was not affected by drought even in the months of low rainfall. According to Matos et al. (2014a), a succulent stem acts as a water buffer that delays dehydration in months with low water availability. Maintaining leaf hydration is important for the absence of a variation in leaf chlorophyll concentration. The constant leaf concentration of carotenoids indicates the absence of abiotic stress, since these pigments act in the photoprotection of the photosynthetic apparatus. As a result, climatic conditions, such as low temperatures and rainfall influence production. Liang et al. (2007) report that low temperatures cause plant stress which, in turn, affects several physiological processes. When temperatures drop below 10 °C and thermal amplitude is higher than 10 °C, the leaf senescence process accelerates (Matos et al. 2012). The physic nut is sensitive to low temperatures, and under this condition plant growth declines, remobilizing the nutrients used in this process for storage in organs other than the seeds (Matos et al. 2012). The low temperature period and leaf fall occur after the fruit harvest and, in the absence of strong sinks, the plant can use assimilates to generate greater latex production. Thus, the findings indicate that low temperatures and leaf senescence are directly related to latex production and possibly associated

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with the storage of reserves remobilized from leaves on the stem. Research on *J. curcas* L. conducted separately and independently in different parts of the world show high species sensitivity to cold and frost (Ackerly et al. 2000; Matos et al. 2012). These results differ from those presented by Lima et al. (2002) for the rubber tree (*H. brasiliensis*), showing that environmental factors occurring in different periods, specifically higher average rainfall and temperatures, affect the flow and biosynthesis of latex for cultivation, leading to increased production.

Crown diameter reflects the size of the foliar apparatus that performs photosynthesis. This is in line with the assertion that crown diameter is directly proportional to the number of leaves. Thus, fruit development (priority sinks) depends on the number of source leaves in full photosynthetic activity. In this condition latex production is a secondary sink inversely correlated with crown diameter and fruit production. Multiple regression analysis (Table 3) demonstrated that latex production is positively associated with plant height, possibly because of the long stem with greater storage capacity. It is known that during the fruiting process the plant uses most assimilates to develop the fruit and a lesser amount to produce secondary compounds, resulting in lower availability of these metabolites for latex production (Peres 2004). Moreover, it is important to underscore those inflorescences and physic nut fruits are produced at the tip of branches (Saturnino et al. 2005) and thus latex and fruit production compete for the same resources arising from photosynthesis. As such, latex production is negatively correlated with fruit production and other variables that maximize it.

In general, a comparison between average physic nut latex production and the average production of other lactiferous plants of the same family, such as the rubber tree (*H. brasiliensis*), demonstrates that physic nut latex production is much lower. Whereas the rubber tree clone GT1 has a mean production of 61.97 mL/

collection/plant and the RRIM 600 variety a production of 53.79 mL/collection/plant (Kshirsagar 1999), our results exhibited a mean production of 1.00 mL/collection/plant. This low yield limits the commercial exploitation of physic nut latex by the pharmaceutical industry.

One characteristic that could explain the significant difference in latex production between species of the family Euphorbiaceae is the morphology of lactiferous plants. In the rubber tree, a species with high latex production, lactiferous vessels are articulated (Mesquita and Oliveira 2010). In *J. curcas* L., a plant with low latex yield, there are different types of lactiferous vessels: articulated anastomosing and non-articulated lactifers, and non-articulated branched and non-branched lactifers (Huan-Fang et al. 2006). Furthermore, the number of lactiferous vessels and their location influence species productivity. In the rubber tree, lactiferous vessels are present in all plant organs, while in the physic nut they are found in larger numbers in the phloem and cortex (Mesquita and Oliveira 2010; Huan-Fang et al. 2006). In addition, the physic nut is an oleaginous plant that produces a high percentage of seed oil (35%) and the production of oil, seeds and latex results from intense competition for the same resources arising from photosynthesis.

CONCLUSION

Our data indicate that the latex production from *J. curcas* L. is influenced by the climatic conditions such as low temperatures and rainfall. In addition, the latex production is inversely correlated with crown diameter and fruit production, and positively associated with plant height. Based in our results, it is recommended to perform the latex extraction in post-harvest fruit periods. The present study showed that the low latex productivity is a limitation for its use on pharmaceutical industry.

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