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AGE AND GROWTH AFFECT OLEORESIN YIELD FROM COPAIBA TREES IN THE CERRADO-AMAZONIA ECOTONE

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HIGHLIGHTS

Oleoresin production of *Copaifera* spp. under natural environment is dependent of the age and growth.

The methodology can be applied for the individual selection in natural environment.

Oleoresin production is variable in natural environment.

ABSTRACT

Copaiba trees, of the genus *Copaifera* (Fabaceae), produce an oleoresin that has pharmacological applications. The yield from the trees is very variable, and factors affecting production are still unknown. We evaluated the yield of oleoresin from *Copaifera* spp. in the Cerrado-Amazonia ecotone, as well as its relationship with the growth and age of trees. We sampled 30 *Copaifera* trees by extracting oleoresin for 24 h with a metal borer. Increment cores were collected from 15 trees to determine their age by counting growth rings and to calculate the mean annual increment of the diameter at breast height. The cylinders were sanded and the number of growth rings was counting with a magnifying glass (10x). The ages of trees from which increment cores were not collected were estimated by simple regression analysis. The proportions of productive and non-productive trees were recorded. The best adjusted model for age estimation showed $R^2_{\text{adjust.}} = 0.616$ and $S_{yx}\% = 4.42$. The average productivity of oleoresin was 0.124 L per day, and 30% of the trees were productive. The proportion of productive trees increased with increasing diameter at breast height and age, but after a point, increasing diameter was associated with reduced productivity. The mean annual increment had an inverse relationship with diameter for the productivity of oleoresin. The results suggest that other factors could be triggering and controlling the oleoresin production and not only the age.

Keywords:

Copaifera spp
Diameter at breast height
Mean annual increment
Sustainability

Historic:

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INTRODUCTION

Copaiba trees, 46 species which belong to the genus *Copaifera* (Fabaceae), are found in the tropical regions of South and Central Americas, West Africa and maybe Asia (Martins-da-Silva, 2006). According to Martins-da-Silva et al. (2008), there are nine distinct species in the Amazon region of Brazil, including some species that have a range of physical characteristics, which makes correct identification difficult.

Copaiba trees produce an oleoresin with multiple applications, which is also used as a source of bioactive molecules in pharmacological applications (Veiga Junior and Pinto, 2002). It is a byproduct of the excretion or detoxification of the tree that is used as defense against animals, fungi, and bacteria when exuded by slits in the decaying wood (Alencar, 1982; Plowden, 2003). Oleoresin has been used to treat a number of airway diseases and respiratory, dermal, and mucosal infections in humans, and it has aphrodisiac, anti-tetanus, anti-rheumatic, anti-headache, and anti-venom properties (Veiga Junior and Pinto, 2002; Pieri et al., 2009). It is used as a raw material in creams, shampoos, and soaps in the cosmetic industry and in varnishes, lacquers, paints, perfume fixatives, and papermaking (Ferreira and Braz, 2001).

Factors that hamper its commercial exploitation are the high variation in the yield of oleoresin by copaiba trees and the limited proportion of productive trees in a population. Although some studies have tried to explain the factors associated with copaiba production, uncertainties still remain (Alencar, 1982; Baima et al., 1999; Ferreira and Braz, 2001; Rigamonte-Azevedo et al., 2004; Oliveira et al., 2006; Rigamonte-Azevedo et al., 2006; Medeiros and Vieira, 2008; Barbosa et al., 2009; Newton et al., 2011; Martins et al., 2013; Souza and Higuchi, 2014). Tree growth is related to oleoresin yield, and physiological conditions are expected to influence its production (Plowden, 2003).

Dendrochronology is a powerful science to study the dynamics of tree growth (Vlam et al., 2014). Based on the age distribution and the characteristics of the tree rings, inferences can be made to support sustainable forest management (López et al., 2013; Vlam et al., 2014).

The region between Amazonia and the Cerrado in Brazil is experiencing high rates of forest exploitation and deforestation for economics activities. However, oleoresin is a potential alternative product in the region that could generate an economic return without causing deforestation. In this sense, the aim of the present study was to evaluate the yield of oleoresin from *Copaifera* spp. in a primary forest in the Amazonia-Cerrado ecotone and to determine the relationship between oleoresin yield and tree age and growth, in order to evaluate whether copaiba could be sustainably harvested in the region.

MATERIAL AND METHODS

Study area

The study was performed in a 213-ha area of a primary forest located in the Legal Reserve Area of a private property in the Municipality of Vera, in the central north region of the State of Mato Grosso, Brazil (Figure 1). An inventory of the forest was conducted in 2011 for logging purposes, although the area contains species that have potential as non-timber resources as well. The geographical coordinates of the area are 12°42'54" S and 55°19'53" W (Datum WGS84, 21S), and the altitude is around 330 m above sea level.

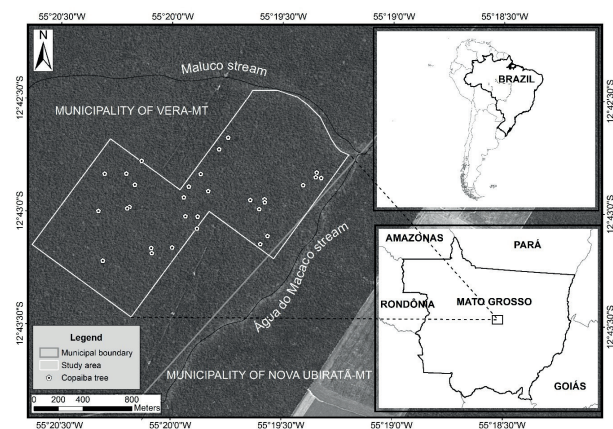


FIGURE 1 Localization of the study area in the Cerrado-Amazonia ecotone in the central north region of Mato Grosso State, Brazil.

The region is located in the watershed of the Amazon River. The vegetation is classified as lowland semi-deciduous seasonal forest with an emergent canopy and characterized by species from the Amazon, and overlaps with both the tropical rainforest and the Cerrado (Cerrado-Amazonia ecotone) (Ibge, 2012). The typical climate during most of the year is humid, with only three dry months, a maximum temperature above 18°C across the year, and an annual rainfall of 1850 to 2050 mm (Köppen classification Aw) (Inmet, 1992).

The soil is classified as dystrophic Yellow Red Latosol plinthic and has a typical medium texture, with a low natural fertility and contains excess aluminum. The relief, formed by the accumulation of sediments over the years, is known as Parecis Plateau (Ibge, 2009a; 2009b; 2009c).

Data collection

The forest inventory of the study area for forest exploitation (census of the trees with ≥ 0.25 m diameter at breast height (DBH); unpublished data) identified 72 *Copaifera* trees (2.95 trees ha⁻¹), of which 30 were

selected for the present study based on their DBH. These trees had a DBH greater than 0.30 m and were selected because trees with lower DBH do not produce oleoresin (Plowden, 2003). The trees were also selected to create a sample that was distributed across DBH values and ages.

There were seven samples in the 0.30–0.42 m DBH class, six for 0.42–0.54 m, 11 for 0.54–0.66 m, and six for 0.66–0.78 m. Before oleoresin collection, the circumference at breast height (CBH) of the trees was measured again with a tape measure. The values were converted to DBH using the following formula:

$$DBH = \frac{CBH}{\pi} \quad [1]$$

The extraction of oleoresin from the *Copaifera* trees was performed during timber harvesting activities in the study area, from July through September 2012, the dry season in the region. For the extraction, a hole was bored at breast height using a 1.91-cm metal borer. The hole's location was below the main branch or on the side toward which the trunk was inclined.

After we had bored to the center of the trunk, often surpassing the pith, we inserted a plastic hose in the hole and connected it to a 5-L plastic barrel, affixing it with adhesive tape. Even in trees that did not produce oleoresin during the boring, the hose connected to the barrel was left inserted for 24 h, following the methods of Rigamonte-Azevedo et al. (2006). After oleoresin collection, the hole was sealed to protect the tree against possible attacks from pathological organisms. The sealing was performed with branch wood near each tree; the wood was cut and debarked with a machete.

The oleoresin obtained was stored in barrels marked with the identity of the trees and subsequently transferred to a laboratory for volume measurement in test tubes with a milliliter scale. The trees were considered productive if the amount of oleoresin extracted leaked out of the barrel into the test tubes and non-productive if the quantity was not sufficient to flow out and the oleoresin remained in the inner surface of the pipe or barrel.

Individual age was estimated by counting the number of growth rings, which are marked by parenchyma cells in copaiba trees (Figure 2) (Mainieri and Chimelo, 1989). According to Pereira et al. (2018), there is a high correlation between growth rings in copaiba populations, whose series is strongly influenced by environmental factors. We sampled 17 trees for age determination; one wood increment core perpendicular to the hole for oleoresin extraction were removed after complete exudation using an increment borer. Two of

the 17 trees were hollow and yours increment cores were discarded. The holes made by the increment borer were sealed in the same manner as the holes for oleoresin extraction. To allow better visualization of the anatomical structures, the samples were sanded using sandpaper with 100, 150, 220, 320, and 400 grains of sand per cm² and then the number of growth rings was counted, using a magnifying glass (10×).



FIGURE 2 *Copaifera* spp. and habitat, and the *Copaifera* spp. growth rings in the Cerrado–Amazonia ecotone in the central north region of Mato Grosso State, Brazil.

Processing and data analysis

The age of the 15 trees from which no wood cylinder was collected was estimated using linear regression analysis. In regression analysis, samples with fewer than 30 observations are appropriate for analysis only by simple regression using an independent variable with strong correlation (Hair et al., 2009). For this analysis, the age (dependent variable) was estimated on the basis of its relationship with the DBH (independent variable).

Prior to fitting the model, we evaluated the variables to verify the statistical assumptions of normality (Shapiro–Wilk test, $\alpha = 0.05$), linearity, and homoscedasticity (scatterplot analysis), eliminating the possibility that the relationship was misrepresented (Hair et al., 2009). To improve the correlation between age and DBH, the variables were transformed before the models were adjusted.

Four equations were selected for the residual plot analysis to obtain the highest adjusted coefficient of determination ($R^2_{\text{adjust.}}$), lowest standard error of estimate ($S_{yx} \%$), and significance according to the F test, as well as the estimated coefficients according to the *t*-test (both at a level of 5% probability).

The mean annual increment (MAI) was calculated as the ratio of DBH to the individual's age. This variable was used to evaluate if the copaiba trees were in a senescence or growth phase under unfavorable conditions. After we obtained the data for age and MAI of DBH, the proportion of productive trees in the different classes of the studied variables (DBH, age, and MAI) was

verified. The oleoresin yield was used in the descriptive statistical analysis, in which we compared the region's yield (minimum, maximum, and average) and production (productive or non-productive) with those data for other Amazon regions.

RESULTS

The DBH and age variables both showed a normal distribution at the 0.05 significance level (DBH: $W = 0.919$, p -value = 0.189; age: $W = 0.964$, p -value = 0.760) and complied with the statistical assumptions for the simple regression analysis. The scatterplot revealed a low tendency to linearity and homoscedasticity (Figure 3). An atypical observation around 140 years was eliminated to improve the correlation between the variables, which changed the value of the Pearson coefficient of correlation from 0.671 to 0.761. This shows how complex are the growth trajectories of the trees growing up in a such complex forest and the difficulties to estimate the age with the DBH.

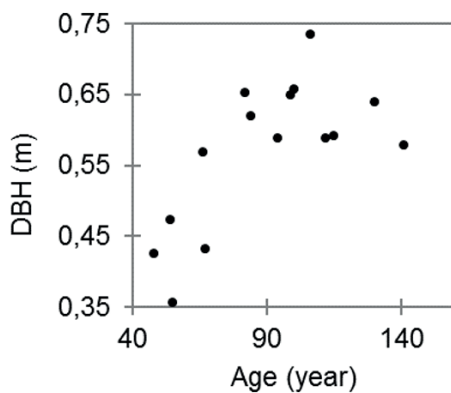


FIGURE 3 Scatterplot for diameter at breast height (DBH) and age of *Copaifera* trees in the Cerrado-Amazonia ecotone in the central north region of Mato Grosso State, Brazil.

Even after verifying that the DBH and age variables complied with the statistical assumptions, the adjustment of the equations showed better results after the inverse, square, root, and/or, mainly, logarithmic transformations of the variables. The best four adjusted equations were selected through the observation of residuals plot, and the results of the intercepts (β_0) and regression coefficients (β_1), adjusted coefficients of determination ($R^2_{\text{adjust.}}$), standard errors of estimate ($S_{yx}\%$), and F test were considered (Figure 4).

Models 1.2, 1.3, and 1.4 showed similar statistical parameters. Model 1.3 was selected to estimate the age and showed superior $R^2_{\text{adjust.}}$ and less standard error of estimate than the other two models. The residuals plots were similar in all the models. Although model 1.1 showed the best $R^2_{\text{adjust.}}$ (0.656), it was discarded because of the high value of the standard error of estimate (19.33%).

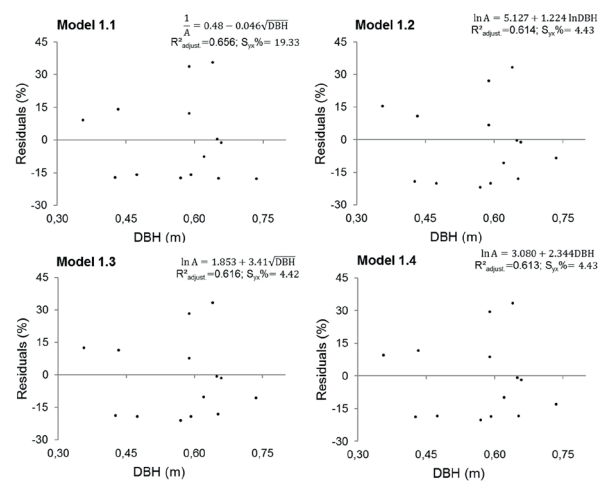


FIGURE 4 Scatterplot for diameter at breast height (DBH) and age of *Copaifera* trees in the Cerrado-Amazonia ecotone in the central north region of Mato Grosso State, Brazil.

Among the 30 copaiba trees selected for oleoresin extraction, only 9 (30%) were productive, including 5 that were hollow and were distributed among all the DBH classes. The average yield of copaiba oleoresin was 0.124 ± 0.079 L tree⁻¹ 24 h⁻¹, with a maximum value of 2.352 L tree⁻¹ 24 h⁻¹. There was a large variation (CV = 349.9%) and distribution out of normality at the 0.10 significance level ($W = 0.314$, p -value < 0.0001; Figure 5).

After defining a regression equation that was adequate for estimating the age of the trees, we evaluated the yield of copaiba oleoresin and proportion of productive trees according to DBH class, age class, and MAI of DBH class (Figure 6). Few individuals with either a small or a large DBH were productive, but there was a high proportion of productive individuals in the intermediate class of DBH (0.54 m to 0.66 m). This proportion was reduced in the largest diameter class (0.66 m to 0.78 m). The Pearson coefficient of correlation between

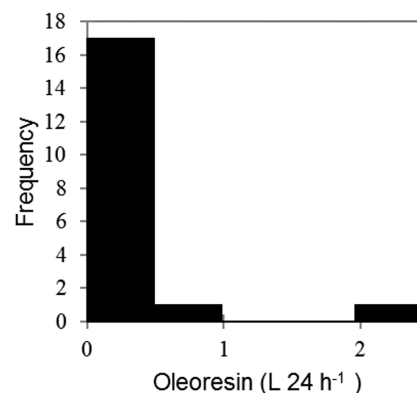


FIGURE 5 Histogram of the yield of *Copaifera* oleoresin in the Cerrado-Amazonia ecotone in the central north region of Mato Grosso State, Brazil.

age and MAI of DBH ($r = -0.77$) indicated a significant inverse relationship between these characteristics (i.e., as the age increases, the MAI of DBH decreases, and the proportion of productive trees increases).

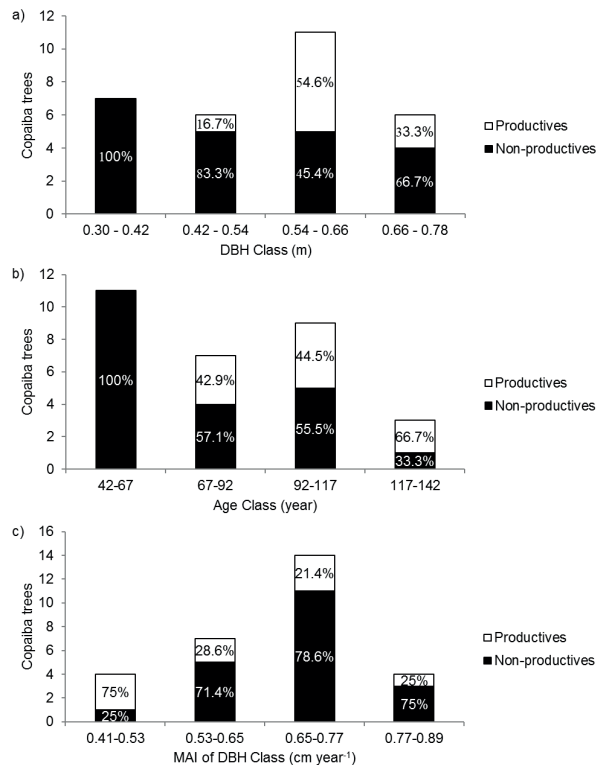


FIGURE 6 Distribution of productive and non-productive *Copaifera* trees according to the (a) diameter at breast height (DBH) class; (b) age class; and (c) mean annual increment (MAI) of DBH class in the Cerrado–Amazonia ecotone in the central north region of Mato Grosso State, Brazil.

DISCUSSION

The heterogeneity of natural tropical forests confers a high diametric amplitude to the trees, which could be why R^2_{adjust} showed a weaker relationship between age and DBH than that observed in studies of forest plantations, in which the diametric amplitude tended to be more homogeneous (Finger et al., 1996; Canto and Schneider, 2004; Schneider et al., 2006; Sanquetta et al., 2010). There are few models that predict tree growth using tree size variables in tropical rainforests (Da Cunha et al., 2016). Therefore, even though R^2_{adjust} was lower than that observed in the adjusted equations for planted forests, the age equation for the copaiba trees in the study was used to estimate the age of the trees for which the variable was not determined.

Results on the relationship between growth and age in copaiba trees provide valuable data to improve the science and management of *Copaifera* (Free et al., 2017). Da Cunha et al. (2016) verified that copaiba trees may have lower growth rates than other Amazonian species. In the Cerrado–Amazonia ecotone, the dry season may limit tree growth, causing these rates to be even lower (Nepstad et al., 2004).

The distribution of productive trees with regard to DBH is similar to the results obtained in other studies, which verified that individuals with smaller diameters were less productive or completely non-productive. The intermediate classes showed higher values for oleoresin yield, whereas individuals in the higher diameter class were less productive or non-productive, as in the lower diameter class (Alencar, 1982; Plowden, 2003; Rigamonte-Azevedo et al., 2006; Barbosa et al., 2009; Souza and Higuchi, 2014).

A similar relationship was also verified by Baima et al. (1999), who observed that copaiba trees with DBH less than 0.60 m did not produce oleoresin, while those with DBH between 0.60 and 0.93 m produced oleoresin in proportional to the increase in diameter. These authors did not mention whether there was a decrease in yield in the trees with larger diameters.

Plowden (2003) showed that very young, very old, or hollow *Copaifera* trees do not produce or produce little oleoresin in their trunks; thus, age and a minimum DBH value are preconditions for selecting trees for extraction. Plowden (2003) also indicated that circumstances in some trees may induce the production of oleoresin above the average value in living tissues during middle age, and a different set of circumstances may lead to the creation of cavities filled with oleoresin in the heartwood at an advanced age.

In the present study, while the number of productive individuals increased in accordance with the age class, the opposite trend was observed with respect to MAI of DBH. This finding can be related to tree growth under unfavorable conditions (e.g., water deficit, toxic elements in the soil, fungus, and pest attack) or to the senescence phase of the individuals mentioned by Plowden (2003). However, further studies should test this hypothesis and evaluate other related factors, considering that the non-productive individuals were also observed that had a superior MAI.

Wide variation in the yield of copaiba oleoresin was also observed in other studies, like that sampled trees in the Brazilian Amazon (Table 1). All of these studies reported that some trees were non-productive,

and the average yield of copaiba oleoresin was within the range reported in this study (Alencar, 1982; Baima et al., 1999; Plowden, 2003; Oliveira et al., 2006; Rigamonte-Azevedo et al., 2006; Barbosa et al., 2009; Newton et al., 2011; Martins et al., 2013; Souza and Higuchi, 2014).

The Amazon region is rich in natural resources and has a great diversity of economically important species. Studying them can improve the management techniques and technologies for the appropriate use of their products and by-products (Baima et al., 1999). The high

TABLE I Summary of the yield of *Copaifera oleoresin* in the Amazon according to the literature. p = productive trees only; cs= clay soil; ss=sandy soil; ul=upland; ll=lowland; y=yellow copaiba; w=white copaiba; b=black copaiba; r= red copaiba.

Author	Site	Species	Min.-Max. (L·tree ⁻¹)	Average yield (L·tree ⁻¹)	Production (%)	
Alencar (1982)	Central Amazonia	<i>C. multijuga</i>	1st extraction (March/June 1977)			
			0-2.85cs	0.23cs	38.6cs	
			0-1.95ss	0.16ss	24.0ss	
			2nd extraction (December/January 1978)			
			0-3.5cs	0.13cs	21.5cs	
			0-1.3ss	0.10ss	28.0ss	
			0-1.37cs	0.04cs	10.5cs	
			0-0.5ss	0.04ss	28.0ss	
			4th extraction (September/November 1979)			
			0-1.57cs	0.05cs	10.5cs	
0-0.23ss	0.02ss	12.0ss				
5th extraction (December 1980)						
0-0.45cs	0.03cs	12.3cs				
0-0.75ss	0.03ss	12.0ss				
Ferreira and Braz (2001)	Western Amazonia	<i>Copaifera</i> sp.	Extraction (October/November 1997)			
			-	0.57	40.9	
				1.36ul 0.12ll	43.7ul 39.3ll	
			Extraction (July/August 1998)			
			-	1.70	72.0	
Plowden (2003)	Eastern Amazonia	<i>Copaifera</i> spp.	Extraction (July/August 1996)			
			-	2.10ul 0.32ll	85.7ul 25.0ll	
			1st extraction (July/August 1996)			
			-	0.071 ± 0.039	31.0	
			2nd extraction (November 1997)			
-	0.007 ± 0.005	36.4				
Extraction (May 1998)						
-	0.007 ± 0.005	38.1				
Rigamonte-Azevedo et al. (2006)	Southwest Amazonia	<i>Copaifera</i> spp.	Extraction (January/February 2001/2002)			
			<i>C. multijuga</i>	0-18	0.94	32.2
			<i>C. reticulata</i>	0-11	1.04	30.0
			<i>C. reticulata</i> w	0-4.5	0.48	30.0
			<i>C. cf paupera</i>	0-4.6	1.33	81.5
			<i>C. reticulata</i> b	0-18	1.14	29.6
<i>C. reticulata</i> r	0-5.5	0.38	24.3			
Barbosa et al. (2009)	Central Amazonia	<i>C. multijuga</i>	Extraction (rainy season)			
			0-2.17	0.33	43.7	
			Extraction (dry season)			
0-0.53	0.03	5.9				
Newton et al. (2011)	Western Amazonia	<i>C. multijuga</i>	Extraction (January/December 2009 and April 2010)			
			<i>C. multijuga</i>	0-4.25	0.51p	55.0
			<i>C. piressi</i>	0-0.03	0.02 p	19.0
			<i>C. guyanensis</i>	0-0.03	0.02 p	9.1
			<i>C. guyanensis</i>	0-1.04	0.14 p	32.8
			<i>C. paupera</i>	0-0.27	0.11 p	16.7
Martins et al. (2013)	Amazonia	<i>Copaifera</i> spp.	Extraction (October 2006 to December 2008)			
			<i>C. paupera</i>	-	-	41.8
			<i>C. reticulata</i>	0-2.93	0.52p	46.7
			<i>C. pubiflora</i>	0-2.76	0.45p	62.1
			<i>C. multijuga</i>	0-5.51	1.70p	31.4
0-1.40	0.66p	18.8				
Souza and Higushi (2014)	Southwest Amazonia	<i>C. multijuga</i>	Extraction from unexplored trees (May/July 2008)			
			0-0.95	0.10	41.0	
			Extraction from re-explored trees (May/July 2008)			
0-3.60	0.16	39.0				
Present study	Cerrado-Amazonia ecotone	<i>Copaifera</i> spp.	Extraction (July/September 2012)			
0-2.35	0.12	30.0				

variability in the productive trees and yield demonstrates the importance of identifying the production factors for copaiba oleoresin. Estimates and production forecasts are essential to define limits to the harvest pressure in order to harvest this non-timber product sustainably (Ticktin, 2004). This is the first study of yield of copaiba oleoresin in the Cerrado-Amazonia ecotone, and for this reason we encourage other studies in the region so that the data they collect may be compared with our findings.

Exploitation of non-timber forests to extract non-timber products or sub-products, as well as for agriculture and livestock rearing, is an alternative that may aid conservation of tropical forests and reduce land use change in tropical forests (Ferreira and Braz, 2001; Machado, 2008). Because of its market potential, especially in the pharmaceutical industry, copaiba oleoresin is highly in demand, both in Brazil and abroad; however, the lack of a good production base makes the commercial exploitation of copaiba difficult (Leite et al., 2001).

CONCLUSIONS

It was possible to estimate the age of copaiba trees in the primary forest on the basis of DBH data by using regression analysis. However, the selection of the matrix trees for oleoresin extraction could not be based only on DBH, as each individual tree experiences different conditions that affect its growth, so the correlation between age and DBH is not as strong as that in homogeneous (artificial) forests. The results suggest that other factors could be triggering and controlling the oleoresin production and not only the age. In the primary forest in the Cerrado-Amazonia ecotone, only trees with a DBH of at least 0.42 m and an age of at least 67 years produce oleoresin. However, the use of DBH to identify productive trees can lead to errors, because the proportion of productive trees with high DBH tends to be lower than that of productive trees in the intermediate DBH class. Finally, high variation in the yield of oleoresin was observed in all classes of the variables studied (DBH, age, and MAI of DBH), including non-productive individuals, with the exception of trees in the lower classes of DBH and age, which were non-productive.

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