

# Change in oleoresin productivity between harvests and variable drillings of a *Copaifera reticulata* natural population in the Amazon

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FOREST MANAGEMENT

## ABSTRACT

**Background:** Many gaps in the *Copaifera* oleoresin yield remain unfulfilled, preventing the sustainable management of this valuable non-timber forest product. This work aimed to characterize a natural *Copaifera reticulata* population in the Amazon rainforest, test different positions and depths in the trees to flow oleoresin, and analyze changes in the productiveness between two harvests ten months-spaced. The study was conducted in a Brazilian rainforest area at the Jari Ecological Station (ESEC) in 2017 and 2018, including 26 trees.

**Results:** The diametric and height distributions evidenced light-demanding and mostly medium-class-diameter trees. The annual increment ( $0.45 \pm 0.003$  cm/year) was average, while the occurrence was rare. The area hosts yielding and unyielding trees, providing an average oleoresin production of 603.60 mL/tree. Oleoresin only flowed by reaching the inner heartwood or the pith. Oleoresin was not fully replenished after ten months, but the first drilling stimulated some unyielding trees to deliver it later. Collecting should focus on medium-diameter trees.

**Conclusion:** The growth and distribution behaviors may challenge *Copaifera*'s sustainable management, which depends on the individual tree mechanisms to provide and replenish the oleoresin.

**Keywords:** Class diameter, heartwood, intercellular channels, non-timber forest product, rare species.

## HIGHLIGHTS

A *Copaifera reticulata* population behavior challenges oleoresin production in Amazon;  
The trees only flow oleoresin when drilled between 3/4 of the radius and the pith;  
Medium-class-diameter trees are the targets for oleoresin harvesting;  
Drilling may turn unproductive trees into productive ones after ten months;

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## INTRODUCTION

Copaifera, the common name of all trees belonging to the *Copaifera L.* genus, produces an oily resinous exudate as a secondary metabolite, one of the main Non-Timber Forest Products (NTFP) traded in popular markets in the Amazon. The oleoresin comprises a volatile sesquiterpene-rich oleaginous phase and a denser resinous phase composed mainly of diterpenes (Campanholi *et al.*, 2022). It has attractive antigenotoxic, analgesic, antibacterial, and anti-inflammatory properties to treat pulmonary diseases (Campos *et al.*, 2021), dermatological infections (Campanholi *et al.*, 2022), oral diseases (Bardaji *et al.*, 2016; Abrão *et al.*, 2018; Santos *et al.*, 2022), cancer (Senedese *et al.*, 2019), and skin wounds (Paranhos *et al.*, 2021). Besides human-treating applications, it is beneficial to heal the wounds of equine species (Lucas *et al.*, 2017) and functions as an insecticide for *Spodoptera frugiperda* larvae (Santos *et al.*, 2016; Almeida *et al.*, 2017). The oleoresin quality widely varies among distinct species of the *Copaifera* genus (Silva *et al.*, 2019).

The nine species cataloged in the Amazonia were *Copaifera duckei*, *Copaifera glycyarpa*, *Copaifera guianensis*, *Copaifera martii*, *Copaifera multijuga*, *Copaifera paupera*, *Copaifera piresii*, *Copaifera publiflora* and *Copaifera reticulata* (Martins-da-Silva *et al.*, 2008). The *C. reticulata* Ducke is one of the most oleoresin-productive species. It yielded a maximum volume of 2,760 mL/tree (Martins *et al.*, 2013). Despite the species, the challenge towards a suitable and sustainable copaifera oleoresin extraction in the Amazon remains understanding how the combined effects lead to collectible flowing volumes, traces, or total absence of oleoresin in each tree.

Regarding diameter, age, and heartwood proportion, a previous study on *Copaifera multijuga* showed that trees belonging to an intermediate diameter class (45 – 55 cm) showed the highest and most commercially suitable oleoresin yields. Besides, trees with small diameters without heartwood did not provide oleoresin (Medeiros, 2018). Oleoresin production also depends on relief and the environment. An investigation at a mining company in Pará evidenced that *Copaifera* trees established on slopes were more productive than those in the valley (Gebara *et al.*, 2016). Another study discovered no significant variations in the oleoresin yield in the different Amazon seasons (Gebara *et al.*, 2016). Other factors, such as the year of the study, the species, the age, and the tree's history (previously bored or not), are essential covariates. The time between boring, and the effect of re-boring on the same tree, can also influence oleoresin production (Martins *et al.*, 2013).

Oleoresin of a never-bored tree is available in the inner region of the stem, the heartwood (Medeiros *et al.*, 2018). This wood portion forms after the death of the parenchymal cells, when it stops performing the active transport of raw sap, starting to function only as support and reserve tissue (Magell *et al.*, 1994). The percentage of heartwood in the wood varies between species and trees, diameter, age, and along stem (Yang *et al.*, 2020). The knowledge of *Copaifera* spp. has progressed slowly and discreetly. Studies relating the oleoresin yield to the wood

anatomy and supporting the species' management are scarce (Guarino *et al.*, 2016; Medeiros *et al.*, 2020), unlike those characterizing and using oleoresin. Many gaps about the factors that affect production for each *Copaifera* species remain unfulfilled. Properly managing the species requires discovering the oleoresin's true productive potential and the time required for trees to produce a volume equal to or higher than that extracted in a first harvest. Any timber or non-timber forest resource extraction must occur slower or equal to the resource's replacement, defining a sustainable exploration cycle. In the case of *Copaifera* oleoresin, the stock is usually exploited once in each cycle.

This work aimed (i) to describe the characteristics of a natural *C. reticulata* population within an Amazonia rainforest area regarding dendrometry, growth diameter, heartwood/sapwood ratio, and tree distribution and density; (ii) to test different positions in the tree and hole depths to flow the oleoresin and relate the yield with the population traits; and (iii) to verify how oleoresin yield and proportion of productive *C. reticulata* trees change between two ten months-spaced harvests.

## MATERIAL AND METHODS

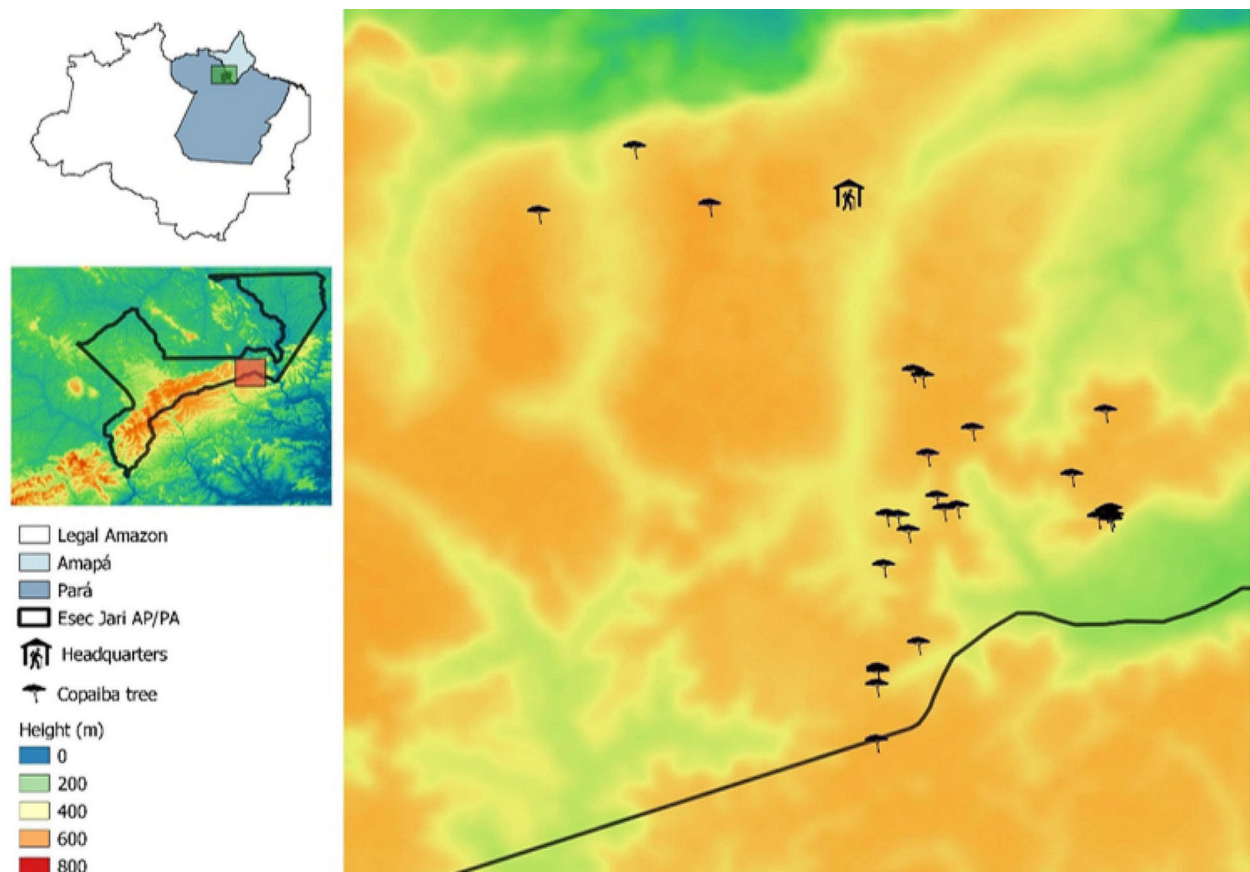
### Study site

The study was carried out at the Jari Ecological Station (ESEC), located in the states of Amapá and Pará, in the municipalities of Laranjal do Jari, Amapá, and Almeirim, Pará, Brazil (Figure 1). The ESEC headquarters, managed by the Chico Mendes Institute for Biodiversity (ICMBio), is located at coordinates 0° 27' 24.18" S; 52° 49' 37.7" W.

The soils are classified as Argisols and Latosols classes, mainly Yellow Latosols and Yellow Red Argisols. Plintosols and Cambisols may also occur. The forest cover in this region is ombrophilous dense submontane, with altitudes ranging from 100 to 600 m, and ombrophilous dense montane, with variations from 600 to 2,000 m (IBGE, 2012), with flat and rugged and sloppy regions. The climate is Am type, characterized by high temperatures and high humidity with monsoons. The average cumulative precipitation in the year is around 2,200 mm. October is the driest month, with rainfall below 50 mm, and May is the wettest, with an average rainfall of 325 mm (Climate data, 2020).

### Inventory of *Copaifera* trees, occurrence groups, and botanical identification

The inventory was conducted in late 2017 and early 2018. All *Copaifera* trees with a diameter at breast height (DBH) above 20 cm were included. Each *Copaifera* had its geographical coordinates registered with a GPS signal receiver. The diameter at breast height – DBH (1.3 m from the ground) was measured with tape, and the commercial height (Hc), with a laser meter. Usually, the shape of the *Copaifera* canopy is elliptical, with thick branches that bifurcate dichotomously. Thus, the canopy area was calculated by the ellipse area considering four radii. A kernel map was generated to show the occurrences groups



**Figure 1:** Location of the Jari Ecological Station, on the state line between Amapá and Pará, in the eastern Brazilian Amazon. The black tree icons represent the *C. reticulata* trees.

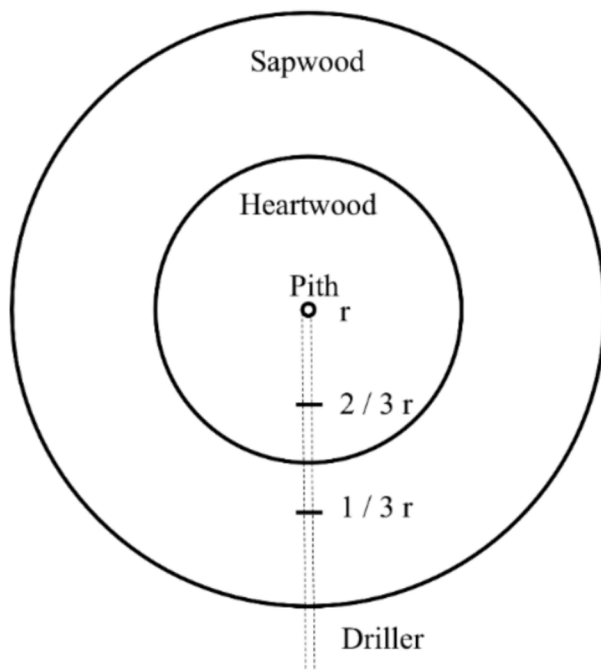
of *Copaifera* formed by the intersection of trees within a radius of 500 meters. The geographical coordinates of each tree were plotted in QGIS 3.12.1, and the inventoried area was calculated to determine the population density. The ICMBio employees named the occurrence groups of *Copaifera* trees as Maran, Road, Gate, and Waterfall. Fertile plant material was harvested for seven *Copaifera* in December 2019, when the flower buds appeared. The material was herborized and sent in duplicate to the Amapaense herbarium - HAMAB for species identification (Collectors: Silva-Junior, J. N. N.; Viana, M. J. de Jesus; and Fernandes, C. collection numbers: 13, 14, 15, 16, 17, 18 and 19).

### Stem oleoresin and wood sample harvest

Among the 28 *Copaifera*s inventoried, only 26 were bored due to a mechanical problem in the drill that prevented drilling two trees. An increment borer (diameter = 1 cm) coupled to a BT 45 Stihl with a gasoline engine and reverse rotation was used. This drill simultaneously extracts the oleoresin and an increment wood core. The two harvests were performed in the low rainfall period (December 2017 and October 2018) between 1.00 and 1.50 m above the ground. Each hole was made with an inclination angle above 15° to handle the drill and the oleoresin drainage. Two holes were

made in each tree to verify the effect of the hole position. The first hole was always made on the shaft's side, where the crown's thickest branch was located (Guarino *et al.*, 2016). The second hole was made on the opposite side and 10 cm above the first hole preventing the holes from meeting in the trunk center. When the oleoresin did not drain from the first hole, the second hole was drilled immediately to check whether pressure release can cause drainage and if oleoresin flow depends on the hole position. When oleoresin drained through the first orifice, the second was done only the next day to not interfere with the drainage from the first one.

Two marks were drawn on the drill to indicate the hole depth in the tree, considering the DBH of the trees. The marks were defined by the stem radius, divided into three depths, each corresponding to a drilling stage. First, it drilled up to 1 / 3 of the radius, then up to 2 / 3, and, finally, up to the total radius, reaching the tree pith if it is not displaced (Figure 2). The workers waited about 1 minute between drillings to ensure no oleoresin flowed. Oleoresin was collected in 2,500 mL plastic beakers immediately after drilling. Next, another container was stuck to the tree, wrapped in foil to protect the oleoresin from light and covered to protect it from rain and dew. The container remained attached to the tree for 24 h. If oleoresin was collected at any time from any holes, the *Copaifera* was considered productive; otherwise, unproductive.



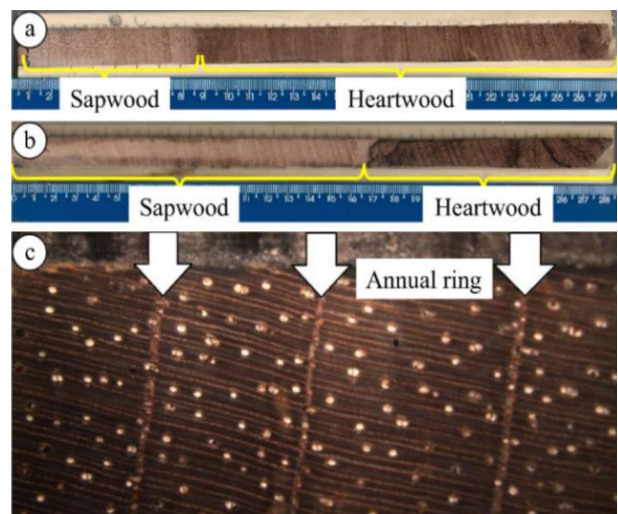
**Figure 2:** Illustrative diagram of the *C. reticulata* tree trunk cross-sections, divided by the hole depth according to the radius ( $r$ ).

The first harvest occurred in December 2017 in the first inventoried *Copaifera*s ( $n = 16$ ), among which one of them already had a hole of unknown precedence. At the end of the harvest, the holes were closed with a wooden stopper. In October 2018, ten months after the first harvest, the wooden stoppers were replaced by a 15 cm PVC tube, isolated with a cap at the end, to facilitate the next harvest. In 2018, 12 additional *Copaifera* trees were found first, but only 10 were drilled because the borer broke. In these trees of the second drilling group, the first holes were closed with tubes and PVC connections, while the opposite holes were closed with an expansive foam of inert polyethylene. In total, 26 trees were drilled between 2017 and 2018, but only 15 trees were sampled two times, e.g., the first and second harvests. The increment cores were packaged in 1 / 2" wide PVC containers for transportation and then room-dried.

### Determination of tree age and the heartwood/sapwood ratio

After drying, the increment cores were glued with white glue on a wooden support, observing the vertical orientation of the fibers to the support. This positioning was essential to allow observation of the cross-section of the wood that shows the annual growth rings. The increment cores fixed on the wooden supports were sanded, with several sandpapers coupled to an orbital sander, in an increasing sequence of granulation (80, 120, 240, 420, and 600). The increment core showed a characteristic shine on the surface, without imperfections, and easily visible rings after sanding.

The increment cores were scanned with maximum resolution (1,200 dpi). The images were uploaded in the ImageJ Pro Plus software version 4.5.0.19 for counting and measuring the lengths of the growth rings in the direction of the pith. The first ring corresponds to the year in which the sample was collected. The quality of cross-dating was evaluated, considering the values obtained from the correlations between the time series of the growth ring and the most extensive time series by Cofecha (Holmes, 1983). After data synchronization, the average cumulative radial increment (CRI) was calculated for each sample, adding the width of each ring to the preceding ring (Costa *et al.*, 2015). Thus, the growth curves of the diameter of each tree were established over time. The average cumulative growth curve of the diameter was constructed from the individual curves as a function of the age of the *Copaifera* tree. The heartwood and sapwood were measured in the increment cores of the first hole (Figure 3).



**Figure 3:** Core samples of *C. reticulata* tree wood, collected in the ombrophilous forest of t Jari Ecological Station, eastern Amazon, showing high heartwood to sapwood proportion (a), low heartwood to sapwood proportion (b) and sapwood regions and the growth ring limits indicated by the white arrows (c).

### Data analysis

The data were tabulated in Microsoft Excel 2013 and exported to the R studio software (R Team Core 2016) for analysis and graphical representation. The association of oleoresin production with quantitative variables was evaluated using Spearman's correlation and regression models. The diameter at breast height was obtained by transforming the circumference breast height (Eq. 1), where:  $DBH$  = diameter at breast height;  $CBH$  = circumference at breast height; and  $\pi = 3.141597$ . The canopy area (Eq. 2) was calculated from the canopy projection radius measurements. Where:  $R_i$  = canopy projection radii;  $C_{area}$  = crown area; and  $\pi = 3.141597$ .



$$DBH = CBH / \pi \tag{1}$$

$$C_{area} = (R1 + R2 + R3 + R4) / \pi^2 \tag{2}$$

The growth curves (Eqs. 3 and 5) were defined based on the Annual Radial Increment (ARI) obtained from the widths of annual growth rings. The annual diametric increment (ADI) was obtained from the radial increment. Where: *ARI* = annual radial increment; *Lr* = width of the ring; *ADI* = annual diametric increment.

$$ARI = \sum_{i=0}^{n=l} Lr \tag{3}$$

$$ADI = ARI \times 2 \tag{4}$$

Data on the widths of the annual growth rings, which generated the annual increment curves, were adjusted using the sigmoidal regression model to assess the diameter-age relationship (Miranda *et al.*, 2018, Schöngart *et al.*, 2007). The following model (Eq. 5) was used to evaluate the different stages of development to establish the ages of the trees from the diametric growth curve. Where: *DBH* = trunk diameter at 1.30 m; *age* = estimated tree age; and *a*, *b*, and *c* are the regression parameters.

$$DBH = (a/(1 + (b/age)^c)) \tag{5}$$

The heartwood/sapwood ratio was calculated following Evangelista (2007), adapted for linear measurements taken directly on the increment core. As the length of the increment core is equivalent to the circumference radius, the length was multiplied by two to obtain the diameter value. Where: *Hw<sub>A</sub>* = heartwood area in the cross-section at breast height; *As* = total cross-sectional area at breast height; *Sw* = sapwood area in cross section at breast height; *Sw%* = sapwood percentage; *Hw%* = heartwood percentage; and *Hw/Sw* = heartwood/sapwood ratio.

$$Hw_A = \pi * (D - 2A)^2 / 40000 \tag{6}$$

$$As = \frac{\pi * D^2}{40000} \tag{7}$$

$$Sw = As - Hw_A \tag{8}$$

$$Sw\% = (Sw/As) \times 100 \tag{9}$$

$$Hw_A\% = 1 - Sw\% \tag{10}$$

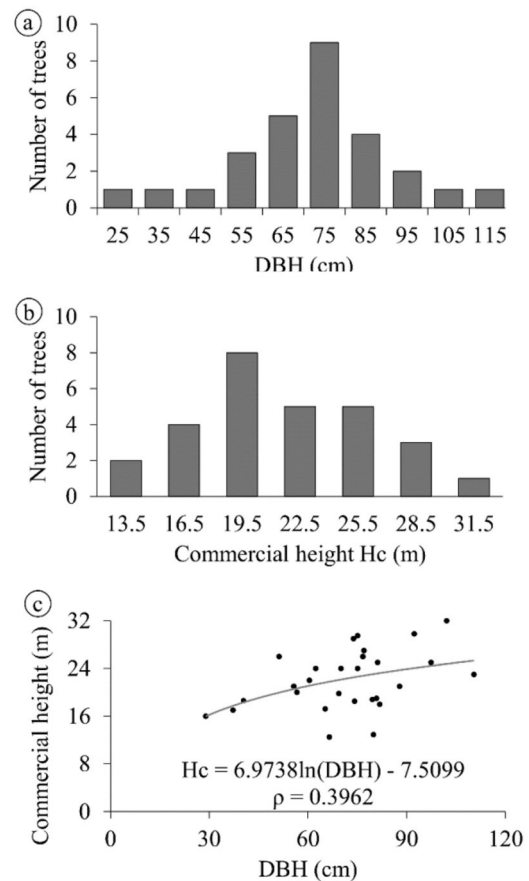
$$Hw/Sw = Hw\%/Sw\% \tag{11}$$

## RESULTS

### Population characteristics

The diametric distribution of the inventoried *Copaifera* trees involved 10 diameter classes, with an amplitude of 10 cm in each category, ranging from 20 cm to 120 cm (Figure

4a). The distribution of commercial heights involved seven classes, with an amplitude of 3 m. Most trees gathered in the intermediate classes (Figure 4b). Spearman's correlation between DAP and Hc was  $\rho = 0.3962$  (Figure 4c).

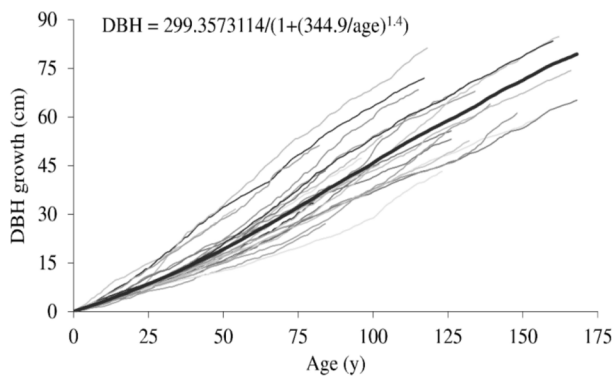


**Figure 4:** Dendrometric patterns of *C. reticulata* population inventoried in the Jari Ecological Station, eastern Amazon: distribution of the diameter class (a, n = 28), commercial height (b, n = 28), and the relationship between DBH and Hc (c, n = 24).

The average annual increment in diameter was  $0.45 \pm 0.003$  cm/year. The estimated age of the youngest *Copaifera* was 54 years, and the oldest 168 years (Figure 5).

Among the attributes of *Copaifera*, the canopy area stood out with the highest number of significant correlations. It correlated well with DBH, age, and heartwood/sapwood ratio. Age positively correlated with DBH and the heartwood/sapwood ratio. It was impossible to estimate the age of two trees; hence, they were removed from the correlation analysis, remaining 24 *Copaifera* trees (Figure 6).

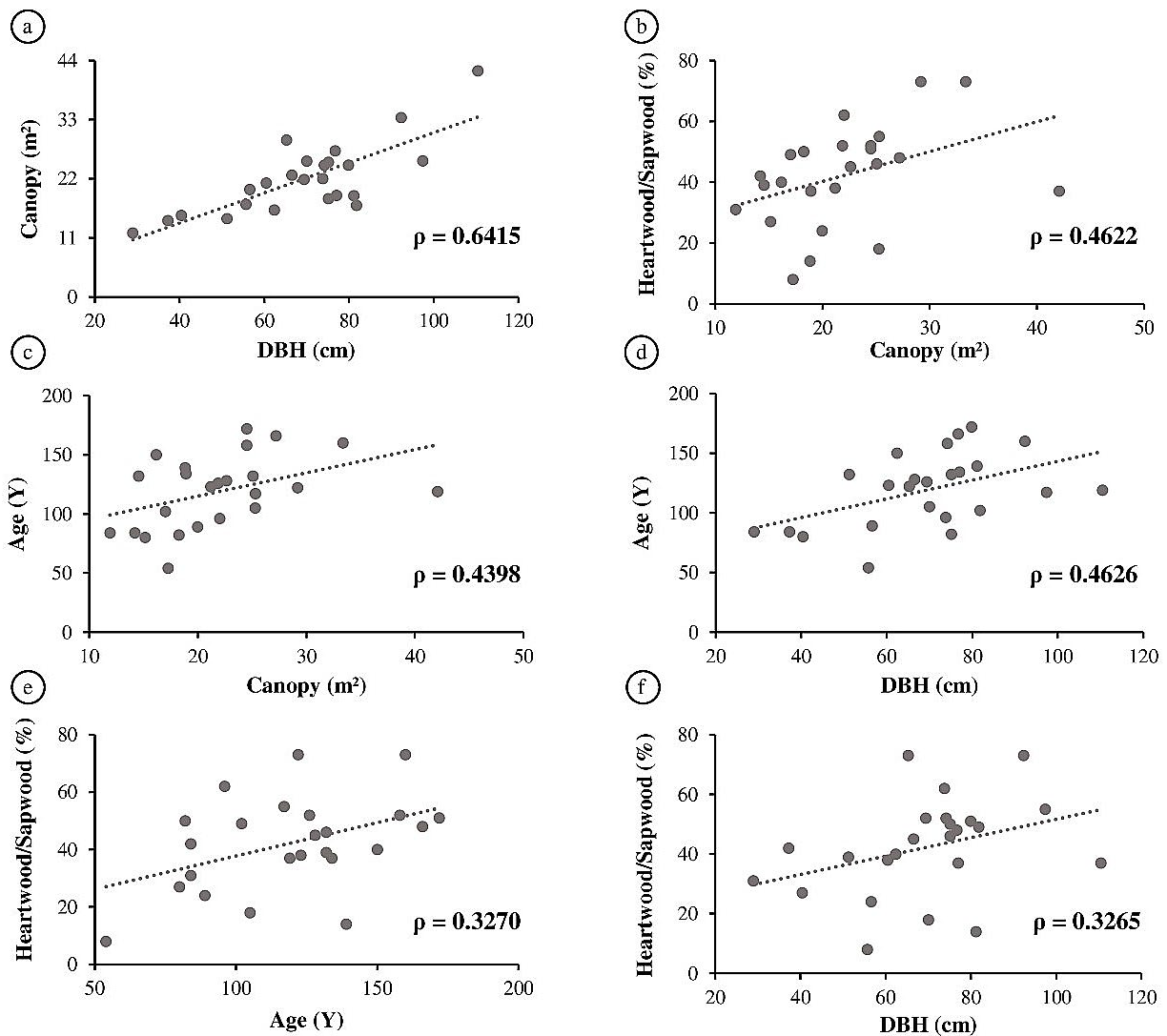
*Copaifera* trees formed four occurrence groups, with eight individuals in Maran, twelve in Road, five in Gate, and three in Waterfall. The three *Copaifera* trees at Waterfall were the farthest (Figure 7). The total area inventoried was 893.43 ha. The overall density was 0.03 *Copaifera* trees/ha. The density of the Maran and Road groups was 0.05 and 0.04 *Copaifera* trees/ha, respectively. In the Gate and Waterfall groups, there were 0.02 *Copaifera* trees/ha.



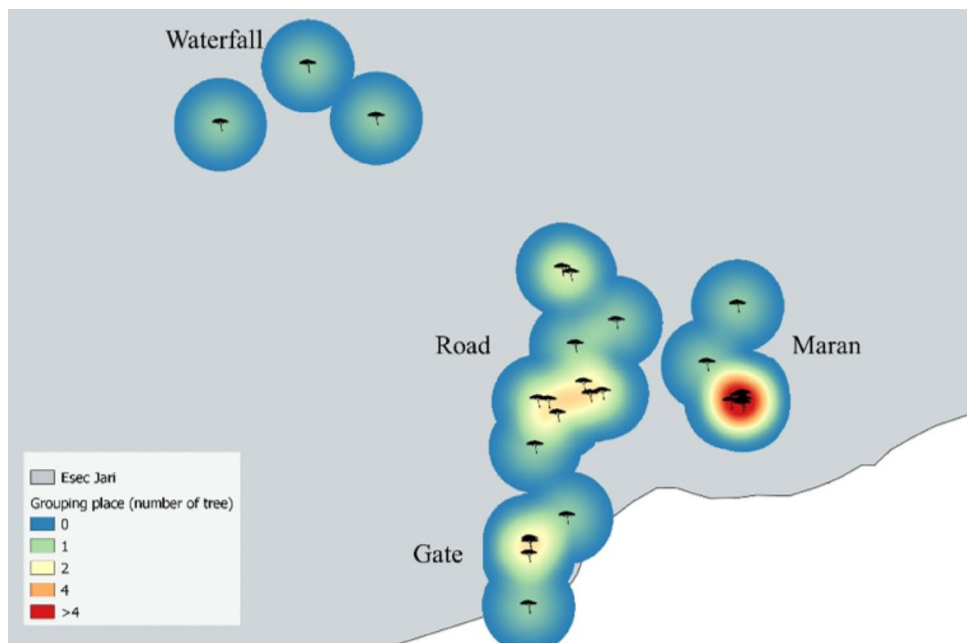
**Figure 5:** Diameter growth curve of *C. reticulata* trees (n = 24) in the Jari Ecological Station, eastern Amazon. The black line represents the model average growth curve used by Schöngart (2007).

### Oleoresin productivity and drilling heights and depths

None of the *Copaifera* trees that did not produce oleoresin in the first hole produced it in the opposite second hole. When drilling up to 1/3 of the radius of the trunk, none of the trees yield oleoresin either. When the hole reached 2/3 of the trunk radius, only three trees started to drain, with a variable flowing rate. The remaining yielding trees only started to flow the oleoresin when the hole reached the region between 2/3 of the radius and the pith, with 100% certainty that the heartwood was reached (Table 1). Most *Copaifera* trees (n = 15) had a linear length of the sapwood of less than 1/3 of the radius. Also, some *Copaifera* trees only started to yield when the drill reached the region very close to the intact pith. Nevertheless, the drill showed smelly oleoresin traces in some trees drilled in the 1/3 and 2/3 radius depths, but not enough for draining.



**Figure 6:** Main correlations (n = 24) between the traits of the *C. reticulata* population of the Jari Ecological Station, eastern Amazon: DBH and canopy area (a); canopy area and heartwood/sapwood ratio (b); age and canopy area (c); DBH and age (d); age and heartwood/sapwood ratio (e); and DBH and heartwood/sapwood ratio (f).



**Figure 7:** Location and group formation of inventoried *C. reticulata* in the Jari Ecological Station, eastern Amazon. The color intensity indicates the grouping of Copaifera trees within 500 m around each tree.

**Table 1:** Number of trees (2017 and 2018) that produced oleoresin by the first hole, total oleoresin oil per hole depth, and oleoresin production by *C. reticulata* at Jari Ecological Station, eastern Amazon (Average ± Standard error).

First Hole Height	Number of trees per hole depth			Volume (mL)	Mean ± Se (mL/tree)
	1 / 3R	2 / 3R	1R		
1.0 – 1.2 (m)	0	1	4	3,090	618 ±218
1.3 – 1.5 (m)	0	4	3	12,330	1,482 ±560
Volume (mL)	0	5,880	9,540	15,420	-

**Changes in productivity between the harvests and relation with DBH**

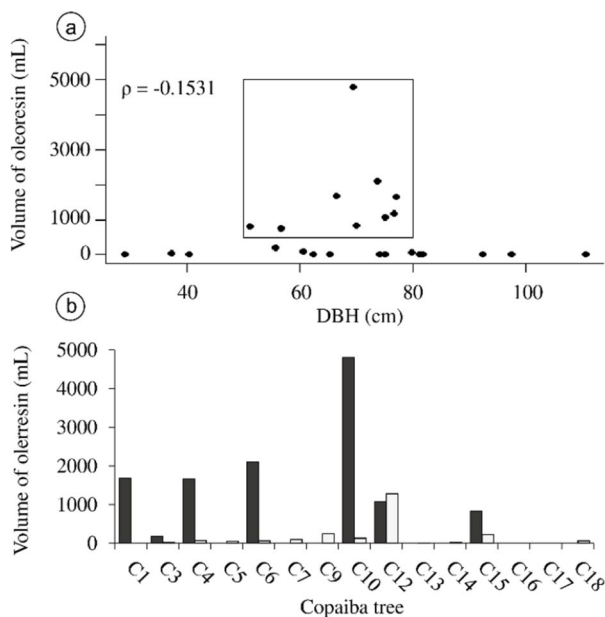
The average oleoresin production by the Copaifera trees in the first extraction, among never-drilled trees, was 603.60 mL/tree. However, the average was 1,257.50 mL/tree, considering only yielding trees were (Table 2).

Spearman’s correlation exhibited a non-significant association between the volume of oleoresin from the first extraction and the biometric attributes of the trees. However, there was a significant relationship (degrees of freedom = 22, Wald = 13.71,  $p < 0.001$ ) with diameter according to the Poisson distribution model, using the log as a link function. The trees of intermediate diameter were the most productive. The minimum volume of oleoresin production (30 mL) was from the tree with the smallest diameter, 37.2 cm, and the maximum volume (4 800 mL) came from a tree diameter of 69.4 cm. The largest diameter of a productive Copaifera tree was 77.0 cm (Figure 8 a).

**Table 2:** Number of first-time bored trees (Total, yielding - Yie., and unyielding - Unyie.), hole that flowed oleoresin and mean oleoresin volume (Vm) drained by Copaifera tree (*C. reticulata*), considering all trees and only productive trees, at Jari Ecological Station, eastern Amazon (Average ± Standard error).

Years	Number of first-time bored Copaifera Trees				All Trees Vm (mL/tree)	Only Productive Vm (mL/tree)
	Total	Yie./ Unyie.	Productive Hole			
			1 <sup>st</sup>	2 <sup>nd</sup>		
2017	15	7/8	7	0	603.6 ±	1,257.5 ±
2018	10	5/5	5	0	208.9	358.4

In October 2018, 10 months after the first drilling, 15 Copaifera trees were revisited to detect new oleoresin production. One of the yielding trees from the first harvest had stopped producing. However, six trees that did not yield oleoresin in the first harvest produced it in the re-harvest. One of these trees was the oldest and placed in the last diameter class. After 10 months, the mean volume of oleoresin was 151.66 mL/tree. Among productive trees, the average was 189.58 mL/tree. The minimum volume was 10 mL, and the maximum was 1,280 mL in the re-harvest (Figure 8). The tree with the highest production (C11) had already been drilled over ten years ago. In December 2017, this Copaifera tree produced 19,000 mL, and in the re-harvest, 1,150 mL. This tree, which showed overproduction, is located along the main road in the most productive Copaifera group.



**Figure 8:** Oleoresin yield correlated with DBH (a) and variation between 2017 (gray bars) and 2018 (white bars) per tree (b). The rectangle marks the *C. reticulata* trees producing above 500 mL in an ombrophilous forest at the Jari Ecological Station, eastern Amazon.

Despite the hole depth effect, there was no significant relationship between the volume of oleoresin and the heartwood/sapwood ratio. However, three *Copaifera* trees were hollow, with wood degraded in the heartwood close to the pith. Thus, three hollow trees (C14, C16, and C30) represented 23.07% of unproductive trees' first harvest ( $n = 13$ ). The C14 tree showed a slight oleoresin production (20 mL), but only in the second collection.

## DISCUSSION

### Population characteristics

The pattern of diametric distribution of *Copaifera* trees is normal. Most of the trees are concentrated within the intermediate classes, with few trees in the first and last classes, indicating an aging population, with little entry of trees in the first diameter classes. *C. multijuga* populations from other sites, such as the slopes of the Saracá-Taquera forest in Pará state (Gebara *et al.*, 2016) and the Tapajós and Paragominas regions (Herrero-Jáuregui *et al.*, 2012) showed similar patterns. Although *Copaifera* trees produce many fruits and seeds, 95.5% of the newly germinated seeds do not develop to the seedling stage (Gebara, 2016). After the rod phase, *Copaifera* trees have high survival rates; but few individuals enter adulthood with  $DBH > 10$  cm due to the high seedling mortality. In the same study, the author observed that with  $DBH > 20$  cm, the population showed decreased establishment rates up to the last diametric classes.

Herein the highest frequency of *Copaifera* trees occurred in classes above 15 m of commercial height. They are dominant and emerging trees in the forest canopy, reaching up to 40 m in total height. Species of the *Copaifera* genus are intolerant to shade (Santos Junior *et al.*, 2004), requiring medium to high light incidence. Although inter and intraspecific competition for light and nutrients, besides seed, seeding, and samplings predation, hinders the establishment of *Copaifera* trees, these obstacles facilitate faster initial growth in height to obtain better luminosity and avoid herbivory. The diametric increment of  $0.45 \pm 0.003$  cm/year of the evaluated *Copaifera* was considered average. The population diametric growth curve adjusted well to the expected theoretical model, showing decreasing rates of diametric increase for older trees and their possible senescence around 150 years old. *C. multijuga* showed similar growth, but senescence occurs by stabilizing the growth curve near 100 years (Medeiros *et al.*, 2018).

Our results indicated that trees with the same diameter likely have very different ages. Some trees grow more than others in unhealthy settlements, even though they belong to the same species. This population trait relates to the intrinsic characteristics of the tree or the influence of the environment, also affecting the heartwood proportion. There is no established pattern in the heartwood to sapwood proportion with the age variation of the *Copaifera* trees. Nevertheless, they are positively but weakly correlated. It is customary to think that trees with the largest diameters have a higher heartwood to sapwood percentage. However, this study showed that the heartwood area varies greatly concerning the diameter at breast height. This relationship is probably related to environmental or genetic factors instead of age or total diameter.

In all groups of *Copaifera* trees formed at the Jari Ecological Station, the tree density was very low, classifying the species as very rare. This result corroborates the population density (0.21 tree/ha) of a *C. reticulata* investigation carried out in the Tapajós and Paragominas regions (Pará State) that reported low abundance and rare occurrence (Herrero-jáuregui *et al.*, 2012). A previous study conducted in Pará State, in the Saracá-Taquera forest, showed density was also low for *C. multijuga* with  $DBH > 10$  cm but with variations between landscapes (Gebara *et al.*, 2015). The authors found densities of 0.55 trees/ha on the slopes and 0.45 trees/ha in the valleys.

Most studies report the species belonging to the *Copaifera* genus as rare due to the species' self-ecology. The low luminosity inside the dense ombrophilous forest at Jari Ecological Station partially explains the low species density in this study. *Copaifera* trees were not observed in this population in the young phase, even though large seedling banks were available after the fruits fell. However, the seedlings do not remain in the environment for long, probably due to intraspecific competition and poor lighting close to the dense forest floor. Many predated seedlings were also observed, further decreasing the probability of establishment. This observation corroborates the findings of Gebara (2016) of over 90% seedling mortality.



## Oleoresin productivity and drilling heights and depths

Regarding oleoresin production, the amount produced seems to be a pattern for the other species of *Copaifera* supported by the literature. In a study that evaluated the production differences between seasons, a mean oleoresin production of 508 mL/tree and a percentage of 43% for unproductive trees were found in the rainy season. The mean was 807 mL/tree in the drier season, with 42% for the unyielding trees (Gebara *et al.*, 2016). Another investigation reported that only *C. pubiflora* presented an average production above 1,000 mL/tree, while only *C. reticulata* showed a percentage above 50% of productive *Copaifera* trees in the first extraction (Martins *et al.*, 2013). These unclear patterns challenge the management and economic viability of the activity since low production is associated with high harvest costs. Moreover, *Copaifera* trees are scarce and far from each other. Thus, it may be more interesting for the forest worker to identify the most productive *Copaifera* trees and concentrate their harvest efforts.

The productive *Copaifera* trees only flowed the oleoresin when the borer passed the sapwood and reached the heartwood or deeper until the pith. Plowden (2003) found similar results since drainage of oleoresin occurred, on average, after introducing 21 cm of the borer, with the mean space between the bark and the heartwood border being 14 cm. The author also observed that in trees with diameters between 25 and 35 cm, whose heartwood corresponded to 5% of the radius, there was no flowing of oleoresin. In trees with diameters over 75 cm, this ratio was higher than 40%. Results found for *C. multijuga* (Medeiros *et al.*, 2018) were also similar. The literature corroborates the theory about *Copaifera* oleoresin availability in the tree: the oleoresin is produced by parenchymatic tissue and transported in the radial plane towards the medulla by radial cells and vessel elements (Medeiros *et al.*, 2020). Production is stored at the heartwood and only moves out by drilling the tree. For this reason, *Copaifera* trees with smaller diameters and early developing heartwood and *Copaifera* trees with larger diameters but with the heartwood already compromised by hollows near the pith will not produce oleoresin.

The *Copaifera* trees in the Waterfall group were the least productive, as they also had the highest proportion of hollow trees. Studies show that hollow *Copaifera* trees do not produce oleoresin (Plowden, 2003; Martins *et al.*, 2013). On the other hand, our results show that at least one hollow *Copaifera* tree (C14), which is not in the largest diameter class, presented oleoresin after being considered unproductive in the first harvest. Therefore, hollow *Copaifera* trees are not unyielding from a physiological viewpoint. Since part of the trunk and the heartwood is compromised, they cannot store the oleoresin that infiltrates the hollow region. This result relates to the tree's age and diameter and supports why the thickest trees are not the most productive (Plowden, 2003; Martins *et al.*, 2013). Trees within the largest diametric classes are more likely to have hollow regions close to the pith, an essential

factor in making decisions for the management of the species because these individuals can be relevant, from an ecological point of view, as seed holders.

No meaningful correlation between the ages of the *Copaifera* tree and the oleoresin production showed up. Medeiros *et al.* (2018) also reported a low correlation between these two parameters. The parameter best correlated with the oleoresin production in the first extraction was the diameter of the *Copaifera* tree. The trees with oleoresin production above 500 mL, which possibly, justify the costs and efforts involved in the extraction, are in the intermediate diametric classes, between 50 cm and 80 cm. These results aligned with other studies on the *Copaifera* genus (Plowden, 2003; Klauberg *et al.*, 2014; Medeiros *et al.*, 2018). An investigation with *C. multijuga* in the Ducke Reserve showed a higher correlation between production and DAP in the first extraction than in the re-collect (Medeiros *et al.*, 2018).

## Changes in productivity between the harvests

The oleoresin volume of the second harvest is underestimated due to problems sealing the holes with the wooden stoppers, as is typically done by forest workers. The instruction of the wooden stoppers caused small cracks that allowed oil to flow through the voids or vessel elements of the wooden stopper. Therefore, quantitative comparisons between the first and second harvests focused on the differences in the number of productive *Copaifera* trees, avoiding the influence of probable loss of oleoresin.

The percentage of productive trees increased in the second harvest because some *Copaifera* trees, previously unproductive, presented resin production in the second extraction. This change corroborates Plowden (2003), who hypothesizes that trees are stimulated to produce resin in response to injury caused by a hole in the trunk since oleoresin is a secondary protection metabolite. The same was observed for the species *Copaifera pubiflora*, which showed a significant increase from two to eight productive trees in the second extraction after 12 months of fallow, contributing significantly to the oleoresin volume in the second extraction (Martins *et al.*, 2013). New production stimulated by the damage caused by the hole explains the increase in the percentage of productive trees. Furthermore, after all the oleoresin drainage, the first extraction may create a pressure difference between the emptied resinous channels. The oleoresin, synthesized by parenchymal cells, is then transported to be stored in these channels and empty vessel elements near the pith (Medeiros *et al.*, 2020). The authors showed that oleoresin is transported on the tangential plane through ray cells and paratracheal parenchyma cells.

Overall, the volume of resin collected decreased in the second harvest after 10 months, as shown in Figure 8. This decrease indicates that the fallow time was insufficient for the trees to produce the same volume removed in the first extraction. Some researchers related the fallow point to a minimum period of 12 months. The results also showed a decrease in the volume produced

in the re-collect for short periods, with few individuals capable of exceeding the first production (Plowden, 2003; Medeiros *et al.*, 2018). Medeiros *et al.* (2018) report observations in two populations of *C. multijuga*. In the first one, production almost doubled concerning the first harvest after four consecutive years (1977, 1978, 1979, and 1980) and fallow of 32 years. In the second population, with two extractions in consecutive years (2004 and 2005) and seven years of fallow, the volume of oleoresin was higher in the last extraction. Still, it did not reach half the volume produced seven years earlier in the first extraction.

The Copaifera trees along the main road (Road) were the most productive. This population is predominantly on the slopes and in a sloping environment, with no signs of flooding during the rainy season. It is in this region the C11 Copaifera tree is located, which presented overproduction. The discovery of this overproductive Copaifera, already drilled in the past and that provided oleoresin volume far above the others (19 L in the 2017 extraction), confirms that a high volume of oleoresin can be replaced if fallow time is respected. Copaifera trees can replenish the extracted volume for more extended fallow periods, presenting possible overproductions, as shown in the field observations with the C11 tree. However, we cannot confirm the rest period between extractions, park rangers who had been working in the area for a long time guaranteed that it was more than a decade ago. However, more long-term and detailed studies are required for each species, involving different fallow intervals, generating a recommendation on the ideal fallow time to maintain productive sustainability in Copaifera tree management.

## CONCLUSIONS

The diametric and height distributions evidenced light-demanding and mostly medium-class-diameter trees. The annual increment ( $0.45 \pm 0.003$  cm/year) was average and did not necessarily reflect tree age. The investigated rainforest area hosts oleoresin-yielding and unyielding Copaiferas. The trees with medium diameters of the normal distribution are recommended as target individuals for sustainable management. An unyielding tree in the first hole is unlike delivering oil by a second hole. Regarding hole depth, oleoresin flow requires reaching the inner heartwood or the pith. Oleoresin yield did not correlate well with tree or wood attributes, showing productivity is dependent on a complex combination of factors. Ten months were not enough to allow oleoresin replenishment by Copaifera trees. Nevertheless, some previous unyielding were stimulated by the hole injury and later delivered oleoresin. The growth and distribution behaviors make Copaifera's sustainable management rather challenging. Thus, the key is understanding the individual tree mechanisms to deliver and replenish the oleoresin.

## AUTHORSHIP CONTRIBUTION

Project idea: JNNSJ, ACLG, LB, MCG

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Funding: ACLG, MCG

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