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Effects of morphological and environmental variation on probability of *Copaifera paupera* oleoresin production

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Abstract - This study evaluated the morphological variation (total and commercial height, and diameter at 1.3 m above ground level - DBH) and environmental variables (slope, distance to water bodies, solar orientation, termites, tree damage and elevation) which influence production of oleoresin by *Copaifera paupera* (Herzog) Dwyer in southwestern Amazon. The present study was conducted at the experimental forest of Embrapa Acre, Rio Branco, Acre State, Brazil. Forty-seven trees with DBH ≥ 40 cm were mapped, of which 10 (21.3%) produced oleoresin immediately after being drilled and 25 (53.2%) produced oleoresin after 5 to 7 days, totaling 35 (74.5%) productive trees. The site slope was the only variable that significantly influenced oleoresin production; the greater the slope, the greater the probability the tree produced oleoresin. The results showed that trees growing in areas with a slope ≥ 10% had 100% probability of producing oleoresin (GLM: $\beta = 0.38$; $P < 0.01$), which can be explained by the tension wood produced in trees growing on slopes and supports the popular belief that *Copaifera* should be drilled on the side of the tree with the largest angle of inclination between the trunk and ground level.

Efeitos da variação morfológica e ambiental sobre a probabilidade de produção de oleorresina por *Copaifera paupera*

Resumo - O objetivo deste estudo foi avaliar como variáveis morfológicas (diâmetro a 1,30 m do solo - DAP e alturas total e comercial) e ambientais (declividade, distância de corpos d'água, orientação solar e altitude) controlam a produção de óleoresina de *Copaifera paupera*, tendo como hipóteses: (i) quanto maior o DAP maior a probabilidade da árvore produzir óleoresina e (ii) árvores próximas a corpos d'água possuem maior probabilidade de produzir óleoresina. O trabalho foi realizado na floresta do campo experimental da Embrapa Acre, Rio Branco, AC. Foram mapeadas e perfuradas 47 árvores com DAP ≥ 40 cm, das quais 21,3% produziram óleoresina no ato da perfuração e 53,2% produziram após vistoria realizada 5 a 7 dias mais tarde, totalizando 74,5% de árvores produtivas. A declividade foi à única variável que influenciou significativamente a probabilidade de produção de óleoresina sendo que quanto maior, maior a probabilidade de produção. Os resultados mostram ainda que árvores em áreas com declividade ≥ 10% apresentam 100% de probabilidade de produzir óleoresina (GLM: β=0,38; P<0,01), fato que pode ser explicado pela formação de lenho de tração em árvores que crescem em áreas com relevo inclinado, suportando a crença popular de que árvores de *Copaifera* devem ser furadas do lado com maior ângulo de inclinação entre o tronco e o solo.

Introduction

Sustainable exploration of non-timber forest products (NTFPs) has been seen as an alternative to Amazon region because it promotes reconciliatory land use and conservation (Fiedler et al., 2008). This vision has stimulated the market for NTFPs, which is reflected in the intense interest of forest managers to produce NTFPs and the diverse studies that have been published since 2000 (Rist et al., 2012). These studies have variously focused on the effects of extraction, the dynamics of forest structure (Wadt et al., 2008; Kainer et al., 2014) or alternatives to improve collectors income (i.e., Duchelle et al., 2014). However, there are few studies about production changes over time of the exploited resources (Gautam & Watanabe, 2002; Klauberg et al., 2014).

Among the more traditional NTFPs that have high potential for sustainable forest management in Amazon are rubber (*Hevea* spp.), the Brazil nut (*Bertholletia excelsa* Bonpl.), *Copaifera* oleoresin (*Copaifera* spp.) and açaí (*Euterpe oleracea* Mart. and *E. precatoria* Mart.). However, there are gaps in what is known about these products. For example, some gaps for *Copaifera* oleoresin production in southwestern Amazon are: (i) improvement of the ability to identify species of the genus; (ii) evaluation of content and composition of oleoresin from trees at different stages of vegetative development; and (iii) development of oleoresin collection systems that indicate the best time and frequency that it should be collected. This last one is justified as the production chain of this product tends to be local showing lacks of infrastructure, technology and government incentives (Santos & Guerra, 2010; Moreira et al., 2011; Newton et al., 2011).

Used since before the arrival of the Portuguese in Brazil, *Copaifera* oleoresin is an important phytotherapy with diverse pharmacological attributes, most importantly anti-inflammatory and healing properties (Veiga Junior & Pinto, 2002). Dean (1996) cited the drug trade of the *sertões* (inhabited inlands of Brazil that, in general, have an arid climate due to their continentality) three centuries after the Portuguese arrived in Brazil, and the oleoresin of *Copaifera* was possibly the most appreciated because of its value as an excipient of dyes, as well as an aid for digestion, and an ointment, supposedly used to cure gonorrhea and elephantiasis. Among all the medicinal plants commonly used by the Brazilian population, *Copaifera* trees can be distinguished due to their innumerable pharmacological applications historically proven by popular medicine. Veiga Junior et al. (2007) studied the chemical composition and anti-inflammatory activity of oleoresin from three species of *Copaifera*. Santos et al. (2008; 2011) and Soares et al. (2013) investigated the activity of four commercial oils form *Copaifera* spp. against *Leishmania amazonensis*. The biological activity and cytotoxicity of diterpenes from *Copaifera* spp. oleoresins were studied by Vargas et al. (2015). The essential oil from *Copaifera* oleoresin is also used by the perfume industry to fix aromas and by the cosmetic industry as an emollient and bactericide in soaps, bubble bath, conditioning creams, moisturizers and hair lotions (Veiga Junior & Pinto, 2002). An increase in demand of *Copaifera* oleoresin, combined with the natural variation of this product and a relative lack of studies about its chemical properties, could compromise the effectiveness of the products (Biavatti et al., 2006).

Since the pioneering publication of Alencar (1982), many studies have been published about the relationship between trees diameter at 1.3 m above ground level (DBH) and oleoresin production (*e.g.* Rigamonte-Azevedo et al., 2006; Martins et al., 2012). The focus on this relationship is due to the intrinsic characteristics of *Copaifera* spp. wood, which has oleoresin secreting channels arranged concentrically and alongside the growth rings (Alencar, 1982; Marcati et al., 2001). The majority of oleoresin is produced in the wood of the trunk (Martins-da-Silva et al., 2008) where there are concentrically arranged axial secretors canals, next to the growth layers, delimited by axial parenchyma bands (Alencar, 1982; Marcati et al., 2001; Martins-da-Silva et al., 2008; Sonsin et al., 2014).

Due to the uncertainty of the DBH *vs.* probability of oleoresin production relationship and with the objective to improve the management of *Copaifera paupera* (Herzog) Dwyer in southwestern Amazon, the goals of these work were to evaluate the effects of morphological variation (total height, commercial height and DBH) and environmental variables (slope, distance to waterbodies, solar orientation, termites, tree damage and elevation) on the probability of *C. paupera* oleoresin production.

Materials and methods

The study was conducted in Embrapa Acre forest area located in Rio Branco, Acre State, Brazil. The area has approximately 800 ha of Open Rain Forest with two floristic variations: (i) with bamboo and (ii) with palms (IBGE, 2012). The climate of the region is Am (Köppen) (Alvares et al., 2013), with an average annual temperature of 24.8 °C and average annual rainfall of 1,947.5 mm.

Copaifera L. (Fabaceae – Caesalpinioideae)

In Brazil, the highest richness of species of this genus is found in Cerrado domain (Brazilian Savannah) where there are 13 species, followed by 12 species in Amazon, 5 species in Atlantic Forest and other 5 in Caatinga (Costa, 2017). In Amazon, species of the genus are associated with the emergent forest layer and grow in *terra firme* forest and along flooded margins of rivers and streams (Alencar, 1982). In Acre, species are found throughout the state but in low densities, between 0.16 and 1.5 plants.ha^{-1} (Rigamonte-Azevedo et al., 2006). Unpublished data show that the density of *Copaifera* in Embrapa Acre forest area is 0.22 trees. ha^{-1} (176 trees). Based on *Copaifera langsdorffii* Desf., Sonsin et al. (2014) described the wood as having well-defined growth layers marked by terminal bands of axial parenchyma with or without normal axial canals that produce oleoresin (Figure 1).

Mapping and measuring *Copaifera* trees

Based on previous forest inventories and searching throughout the study area, we selected 47 trees of *Copaifera paupera* with diameter at 1.30 m above ground level (DBH) ≥ 40 cm (only *Copaifera* trees with a DBH ≥ 40 cm can be managed) which were mapped using a GPS equipped with a high-sensitivity sensor. Commercial tree height (CTH) was calculated subtracting tree crown length form total tree height. Both heights were measured with a digital hypsometer and the DBH was measured with a diameter tape (Table 1). The following qualitative information was also collected: (i) presence or absence of termites on the trunk; (ii) presence or absence of physical damage (e.g., areas lacking bark); (iii) hollow trunk detected; and (iv) internal wood rot, based on the presence of sawdust.

Environmental variation

Slope of the terrain (%), distance from waterbodies (m), solar orientation (degrees) and elevation (m) were based on a digital elevation model generated by the ASTER sensor (ASTER GDEM, 2012), with 30.0 m of spatial resolution. All of the analyses and data extraction were performed using the software QuantumGIS (QGIS Development Team, 2013).

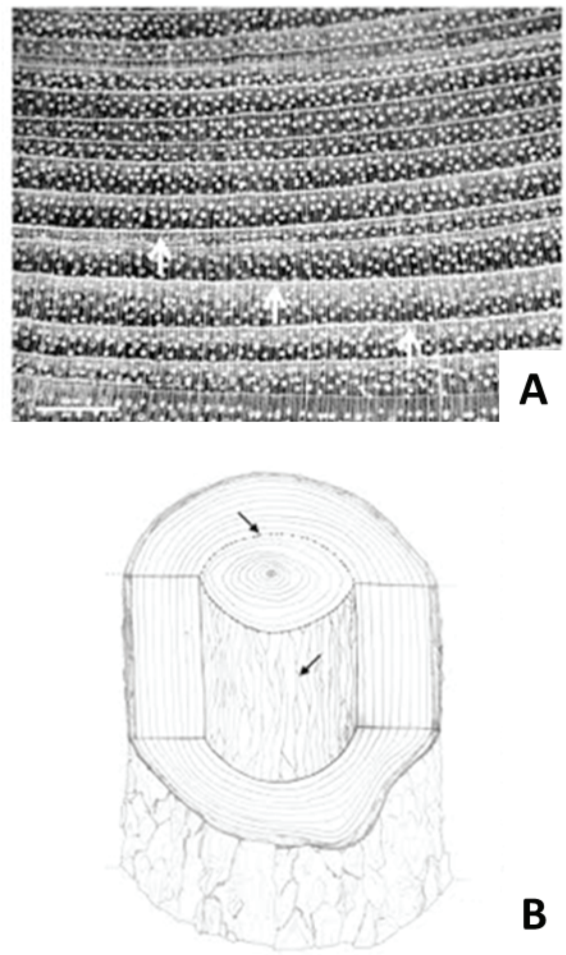

Figure 1. Wood of *Copaifera*. **A**: Cross section, showing growth rings (white arrows) and **B**: Schematic drawing of the trunk, showing the growth layers (in cross section) and the arrangement of the secretory canals (in the longitudinal section, following the growth layer). Credits: **A** - Esemann-Quadros, K. and **B** - Mancinelli, W. S.

Table 1. Descriptive statistics of *Copaifera paupera* morphological variables.

Morphological variables	Descriptive statistics	
	Minimun – Maximum	Mean (± standard deviation)
DBH (cm)	40.9 – 150.9	89.8 ± 25.4
Total tree height (m)	21.0 – 50.8	35.9 ± 6.9
Comercial tree height (m)	11.0 – 29.0	18.7 ± 3.9

Drilling and collecting the oleoresin

The trunk was drilled with a 3/4" auger connected to a chainsaw (Guarino et al., 2016). To collect the oleoresin samples a 1/2" PVC pipe, with a screw lid at one end, was inserted in each hole. The oleoresin was stored in amber glass bottles and sent to the laboratory for further physicochemical analyses. It was not taken all oleoresin from the trees during the first extraction (which allowed the volume produced to be calculated) to guarantee the oleoresin supply throughout the year. The data used here are for the oleoresin collected immediately after drilling and during a second survey one week later.

The drilling procedure was conducted according to the experience of the operator, and was based on a visual analysis of the trunk to decide the best place to drill. The holes were made at a height between 0.70 m and 1.64 m, with a depth that was half the tree diameter. The number of holes varied from one to two per tree; a second hole was drilled if the first did not produce oleoresin. PVC pipes were not put into trees that were hollow or had wood rot. In this case, only a piece of wood was used to plug the hole. The plug was used to protect the tree from insects and other pathogens, and also allowed the tree to heal faster. In addition, if the tree had been previously tapped the existing hole was opened, however if the existing hole did not produce oleoresin than a second hole was drilled.

Statistical analysis

Trees that produced oleoresin (at least 10,0 mL) were analyzed using a generalized linear model (GLM). The response variable used in this study had a binomial distribution (productive *vs.* non-productive trees). A logistic regression with the link function equal to log $(\mu) - \log(1-\mu)$ was used, which is also known as a "logit function" (Kaur et al., 1996). To verify if there was a higher presence of productive trees related to termites or physical damage, the data was grouped into contingency tables and subjected to Pearson's chi-squared test (χ^2).

As predictors of production probability we used DBH, total and commercial height as morphological variables, and slope, distance of the tree to water bodies, solar orientation and elevation as environmental variables. To carry out the statistical analysis, the data for slope, originally expressed as a percent, was transformed using the arc sine formula (√slope) according to Zar (1999). To avoid model overfitting, the variables were selected using a forward-backward selection procedure based on the *small-sample-size-corrected Akaike Information Criterion* (AICc) (Burnham and Anderson, 2002). These authors advocate the use of AICc to evaluate the relative quality of the model when the ratio sample size (n) or number of parameters (K) is smaller than 40 (the study data: $n = 47$, $K = 7$; $n/K = 6.7$). According to this method, the variables are removed and/or added to the model based on changes of the AICc values; the minimum adequate model is reached when no variable can be added or removed without increasing the AICc value.

All analyses were carried out using R (R Development Core Team, 2011) and a significance level of $p < 0.05$.

Results

Thirty-five trees were productive (74.5%). Only 10 (21.3%) yielded oleoresin immediately after the hole was drilled and 25 (53.2%) yielded oleoresin when revisited five to seven days later. Trees with termites and all types of damage recorded (in different combinations) were productive, including trees that were hollow or with internal wood rot (Table 2), however, the results were statistically non-significant (termites: $\chi^2 = 2.18$; d.f. = 1; $p = 0.13$ and physical damage: $\chi^2 = 0.05$; d.f. = 1; $p = 0.81$), which indicated that it was not necessary to use these as co-variables in the logistic regression. The slope was the only variable that significantly influenced the probability of oleoresin production in the study area (Table 3). It showed a proportional relationship where the greater the slope the greater the probability of tree to produce oleoresin. The results show a positive relationship between the slope and oleoresin production, with a higher production probability of trees located in areas with a slope ≥ 10% (Figure 2).

Table 2. Number of productive Copaifera trees by stem damage class.

Damage class	N° of trees	Productive (%)
Sound wood	32	27
Hollow trunk	10	5
Rotten trunk	5	3

Table 3. Summary of logistic regression (GLM with logit link function).

Variable	*β*	Standard error	P (*z*)
Constant	-3.03	0.84	0.18
Slope	0.38	17.52	*< 0.01**

β: regression coefficients, P (z): z statistical significance.

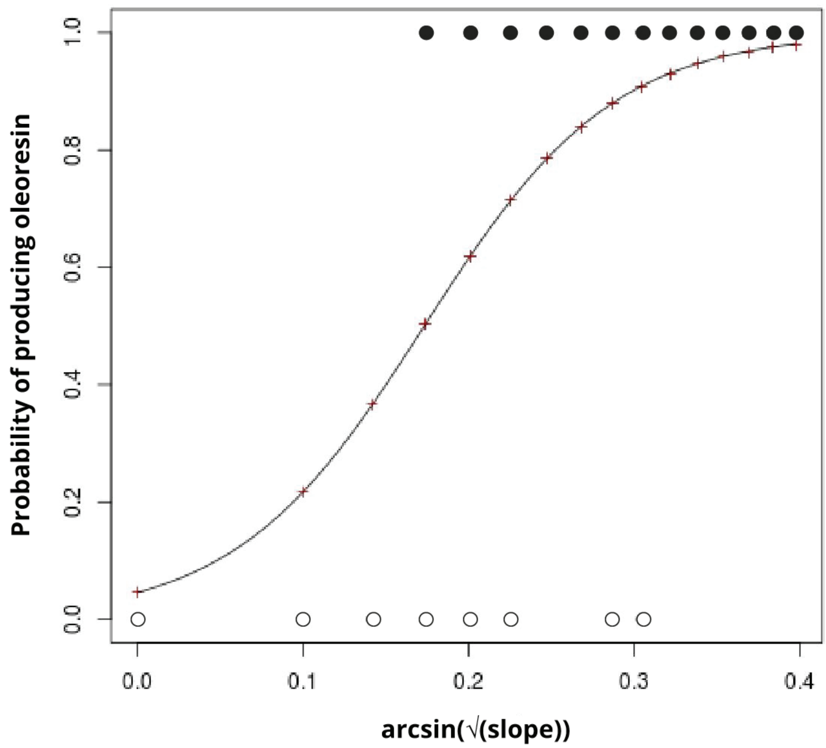

Figure 2. Adjusted curve of *Copaifera* oleoresin production probability in relation to the slope.

Table 4. Summary of extraction season and productive trees (%) of *Copaifera* sp. on Acre State according to the literature. *Only *Copaifera paupera* and *Copaifera krukovii* (Dwyer) J.A.S. occurs naturally in Acre State (Costa 2017), due this the results of Rigamonte-Azevedo et al. (2006) should be carefully considered.

Species	Extraction season	% Productive trees	Author
Copaifera sp.	October/November	40.9	Ferreira & Braz (2001)
	July/August	72	
*Copaifera reticulata** (yellow, white, black and red morphotypes)	January/February	28.5	Rigamonte-Azevedo et al. (2006)
Copaifera cf. *paupera*	January/February	81.5	
Copaifera paupera	October/December	46.7	Martins et al. (2012)
Copaifera paupera	October/November	74.5	Present study

Discussion

The proportion of trees that were productive (>10.0 mL) described in this study is greater than most of the results described in other works conducted in Amazon (Ferreira and Braz, 2001; Medeiros & Vieira, 2008), except the results presented by Rigamonte-Azevedo et al. (2006) that described 81% of *Copaifera* cf. *paupera* trees as productive.

Ferreira and Braz (2001) reported, for Acre State, a proportion of productive *Copaifera* spp. that was 41% for trees tapped during the beginning of rainy season (October/November) and 72% for trees tapped during the dry season (July/August), the latter being very similar to the results of the present study. On the opposite, Martins et al. (2012) suggested that there are more productive trees of *C. paupera* at the end of the rainy season. Unlike these authors, the results of the present study demonstrate that there is not a period when the trees are more productive, because the data analyzed here were collected at the beginning of rainy season (October/November, similar to Ferreira & Braz (2001)) and showed a proportion of productive trees similar to what was described by Ferreira & Braz (2001) and Martins et al. (2012) for the dry season (Table 3). Thus, when excluding interspecific variation from the proportion of productive trees, compared to Martins et al. (2012) and Newton et al. (2011), and the time of the year (dry or rainy season), the data indicate that the higher percentage of productive trees found in the present work was solely due to collecting the oleoresin in two steps (when the tree was tapped, followed by inspecting the tree at a later date if it did not initially produce oleoresin), different from previous studies where tapped trees that did not initially produce oleoresin were plugged and not inspected again. This is supported by the fact that the initial proportion of productive trees was 21.3%, which is lower than what was described by Martins et al. (2012) but similar to results described by Newton et al. (2011) for the same species, on average.

The lack of a relationship between diameter at 1.3 m above ground level (DBH) and oleoresin production may be due to morphological characteristics of the wood. Canals in the same growth layer, or those that are close to each other, can anastomose (Metcalfe & Chalk, 1989) creating a network of canals that produce oleoresin. These anatomical characteristics, of the wood of *Copaifera* species, initially led to the hypothesis that a larger DBH would lead to more secretory canals being perforated when a hole was drilled. However, many authors observed in congeners that not all of the growth layers have secretory canals (Marcati et al., 2001; Melo Junior et al., 2011; Sonsin et al., 2014), which is one of the factors that explains why there is no direct relationship between DBH and oleoresin production. Another important point (in relation to DBH *vs.* oleoresin production) is that DBH accurately represents the environmental conditions that the tree was exposed to throughout its life. Trees that grow on sites with less inter and/or intraspecific competition for resources, mainly light and nutrients, tend to have growth rates that differ from those where there is more competition. Consequently, trees from different sites that have the same DBH can have different number of growth layers (Lobão et al., 2011; 2016) and this may affect the relationship between DBH and oleoresin production.

However, if there are trees that produce more oleoresin, spontaneously and immediately after drilling, while others take hours or days (as observed) or exude less than 1.0 mL, the question is, what is the main variable that influences oleoresin production? Cascon & Gilbert (2000) reported that all species of *Copaifera* produce "oil" or "balm". Therefore, it can be deduced that all *Copaifera* trees produce oleoresin. This is supported by information reported by forest management professionals in Amazon; according to them all *Copaifera* species exploited for timber produce oleoresin in large quantities when the trees are partially or fully cut, which was probably the initial method of oleoresin extraction in this region.

Among the various beliefs related to the production of *Copaifera* oleoresin is that the trees should be tapped on the side of the greatest angle between the trunk and the ground level (Plowden, 2004). Apparently, this study supports this belief because the slope of the terrain was the only environmental variable that affected the probability of a tree to produce oleoresin. A possible biological explanation for this relationship is the formation of tension wood in leaning trees that grow in areas with relatively inclined terrain. In angiosperms, tension wood (TW) is formed on the side of branches (e.g., upper side) and trunks (e.g., when leaning) under tension; for trunks, this is the side with the smaller angle of inclination between the trunk and soil (Metcalfe & Chalk, 1989), resulting in a contraction that pulls the tree to make it stay erect. On the underside, with the larger angle between the trunk and the soil, is the opposite wood (OW). In the TW the vessels generally have a smaller diameter and are less numerous; however, the most striking characteristic is the formation of gelatinous fibers with thicker walls and an inner layer rich in cellulose (called the G-layer), which have microfibrils that are highly crystalline and axially oriented parallel to the cell (Vidaurre et al., 2013). In the OW the wood can have characteristics that are similar to normal wood. Generally, trees that have tension wood exhibit eccentric growth, where the medulla moves from its geometric center towards the side of the trunk that has the largest inclination angle with the soil (Monteiro et al., 2010). In the OW more layers grow in a smaller area of the wood, and because these layers are closer together it is easier for the canals to anastomose. For this reason, more canals are perforated when tapping a tree in this area and the probability of producing oleoresin increases if the OW side of a trunk is tapped for trees growing at an incline.

Although not being evaluated in this paper, the probability of oleoresin production must be associated with genetic variability within and between populations of this species, similar to that occurring with *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. (seringueira; Gonçalves et al. 2006), *Pinus taeada* L. (Roberds et al. 2003), *P. massoniana* D. Don (Liu et al. 2013; 2015) and *P. elliottii* (Lai et al. 2017). In all these species the morphological parameters related to the growth as well as the production of latex (*H. brasiliensis*) and oleoresin (*P. massoniana*, *P. elliottii* and *P. taeda*) showed high heritability, being possible to increase both parameters gradually. In the case of *P. massoniana*, Liu et al. (2015) identified nine genes candidate to regulate the production of oleoresin, among them a gene responsible for pathogens defense response. Chen et al. (2009) estimated to *C. officinalis* that sesquiterpene syntheses production is linked with at least four genes which should be prioritized in future studies.

Conclusions

The probability that a tree of *Copaifera paupera* produces oleoresin in southwestern Amazon is not correlated with DBH, distance to waterbodies, termites, physical damage, solar orientation and elevation. Only the slope of the terrain significantly affected this relationship, which is related to the formation of tension wood in leaning trees that grow in areas with relatively inclined terrain.

In order to increase the number of productive *C paupera* trees, it is recommended that non-productive trees must be revisited after approximately five days. Future studies should be conducted to evaluate the relationship between the number of growth rings and formation of wood with the probability of producing oleoresin in order to test the hypothesis presented in this study and the effects of genetic variability associated with *C. paupera* oleoresin production as well as possible interaction with environment.

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