Traumatic ducts size varies genetically and is positively associated to resin yield of *Pinus oocarpa* open-pollinated progenies

Irenka Fabián-Plesníková[1], Cuauhtémoc Sáenz-Romero[2], Teresa Terrazas[3], Alejandro Reyes-Ramos[1], Miguel Martínez-Trujillo[4], José Cruz-De-León[5], Nahum M. Sánchez-Vargas[1]*

[1] Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Av. San Juanito Itzícuaro s/n, Col. Nueva Esperanza, Morelia, Michoacán, 58337, México.
[2] Instituto de Investigaciones sobre los Recursos Naturales, UMSNH, Av. San Juanito Itzícuaro s/n, Col. Nueva Esperanza, Morelia, Michoacán, 58337, México.
[3] Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, 04510, México,
[4] Laboratorio de Genética y Microbiología, Facultad de Biología, UMSNH, Av. Francisco J. Mújica s/n, Col. Felícitas del Río, Morelia, Michoacán, 58066, México.
[5] Facultad de Ingeniería en Tecnología de la Madera, UMSNH, Av. Francisco J. Mújica s/n, Col. Felícitas del Río, Morelia, Michoacán, 58066, México.

* Corresponding author: NM Sánchez Vargas, E-Mail: nsanchezv@yahoo.com

Abstract

Mexico ranks 5th in worldwide resin production. *Pinus oocarpa* is the most widely tapped pine tree in Mexico. Michoacán (central-western Mexico) is the first nationwide state producer of resin. Despite the *P. oocarpa* relevance, there is no genetic improvement program in the country for resin production. We evaluated the degree of genetic control for growth, anatomical traits, resin yield, and the correlation among them at an early age (five-years-old for growth, six for resin, and anatomical traits) in a *P. oocarpa* half-sib progeny trial. Families were originated from selected mother trees, based on their resin yield. We found significant genetic variation among families for stem volume (h^2_i= 0.12, h^2_f= 0.35), traumatic ducts (diameter: h^2_i= 0.63, h^2_f= 0.51; area: h^2_f= 0.81), and resin yield (individual and family narrow-sense heritability: h^2_i= 0.20, h^2_f= 0.52), and a positive correlation between diameter and area of traumatic ducts with resin yield (Pearson correlation: r= 0.73, p= 0.04; r= 0.71, p= 0.0497; respectively). Results suggest that the early selection (six-years-old) of superior *P. oocarpa* families, based on resin yield (estimated by microchipping technique), and/or based on larger diameter and area of traumatic ducts, appears to be a feasible strategy to develop seed orchards able to provide genetically improved seeds for intensive resin tree plantations. That would be an important alternative for a state as Michoacán, Mexico, where natural *P. oocarpa* stands are being replaced for avocado orchards for exportation.

Keywords: *heritability, open-pollinated families, tapping, defense system, growth, resin ducts, resin yield, diameter, traumatic ducts*

Introduction

Pines are among the tallest and the longest living organisms on earth (Zerbe and Bohlmann, 2014). Due to their large size and longevity, they have been exposed for millions of years to natural enemies as for herbivores and pathogens (Krokene, 2016). Therefore, pine trees have developed complex defense mechanisms to combat these threats (Franceschi et al., 2005). These mechanisms include two defensive strategies: constitutively expressed (always present in the plant) and induced (in reaction to external stimuli) (Moreira et al., 2014).

In the genus *Pinus*, the synthesis of oleoresin (hereafter referred to as resin), a complex mixture of terpenes, is an essential part of its defense mechanism. In addition to its ecological function, mankind has taken advantage of the qualities of resin for a wide variety of uses since ancient times and the practice of resin tapping has been carried out for centuries (Rodríguez-García et al., 2014). It consists of systematically and periodically injuring the tree, causing the secretion of resin and its subsequent recollection (Rodríguez-García et al., 2014). Nowadays, it is a natural source of raw material for various industries (chemical, pharmaceutical, perfume, food, cosmetics, biofuels, and

DOI:10.2478/sg-2022-0002
edited by the Thünen Institute of Forest Genetics

fine chemicals) (Neis et al., 2019a; Vázquez-González et al., 2020).

Resin is synthesized in epithelial cells, which in pine trees are nonlignified, thin-membraned, parenchymal secretory cells that remain functional for longer periods of time than in other coniferous species (Cabrita, 2021). Due to its high toxicity, it is stored under pressure in specialized structures called resin ducts, which are multicellular structures surrounded by layers of epithelial cells (Lewinsohn et al., 1991). Its formation may be a constitutive feature of development in plant tissues and organs (normal axial and radial ducts) or induced by external factors (traumatic ducts) (Pearce, 1996; da Silva Rodrigues-Corrêa et al., 2013; Vázquez-González et al., 2020; López-Villamor et al., 2021). The traumatic ducts (TDs) in the wood of conifers can be radial (longitudinal) or axial (transverse), and unlike typical ducts, they can be found in all (Lin et al., 2002). The main anatomical difference between axial TDs and constitutive ducts in Pinaceae is that traumatic ducts generally form a tangential band (Panshin and de Zeeuw, 1980). That means that more than two canals are side by side, forming a protective barrier to prevent damage to the vital functions of the tree. The formation of TDs is believed to increase resin yield and flow (Ruel et al., 1998; Lombardero et al., 2000) in response to injuries caused by frost, mechanical, chemical, pest or pathogenic damage (Cabrita, 2021). In *Pinus*, in general, inducibility of resin ducts and the TD traits in pine trees have been studied very little (seven species) (López-Villamor et al., 2021).

In Mexico, resin commercial-scale exploitation has been carried out since the last century, mainly in the states of Michoacán, Jalisco, Mexico, and Oaxaca (Francisco-Arriaga et al., 2011). In the State of Michoacán, central-western Mexico, resin tapping is one of the most important non-timber forest resources (Francisco-Arriaga et al., 2011), and the municipality of Ario is the main resin-yielding region of *P. oocarpa*. *Pinus oocarpa* is known to be the most common pine species in the southern half of Mexico and Central America (Dvorak et al., 2009; Lara-Chavez et al., 2011), and it is one of the most tapped species in Mexico (Coppen and Hone, 1995). Even though *P. oocarpa* is listed among the prioritized tree species for Mexico, and it has a high economic, social and ecological value for the indigenous forest communities, there are few breeding practices reported in Mexico that aim at increasing its resin yield capacity (López-Upton et al., 2011). Thus, most of the seedlings used for afforestation with *P. oocarpa* in Mexico have been grown from seed material of mostly unknown origin and without any genetic improvement.

Several studies have shown that resin yield is a heritable trait (Lai et al., 2017; Roberds et al., 2003; Westbrook et al., 2013; Lai et al., 2020; Vázquez-González et al., 2021). Other studies suggest that resin duct traits are importantly associated to productivity of pine resin species (Schopmeyer et al., 1954; Boschiero and Tomazzello-Filho, 2012; Rodríguez-García et al., 2014; Neis et al., 2019b). However, heritability estimates of resin yield and its association to resin ducts are not yet available for *Pinus oocarpa* in Mexico.

Previous studies by our research group, with the same open-pollinated progenies studied on this paper, have explored genetic variation in growth traits (Fabián-Plesníková et al., 2020), and resin yield of the *P. oocarpa* progenies' mother trees (Reyes-Ramos et al., 2019), although heritability was not assessed on the later. In addition, we quantified constitutive resin duct morphological characteristics in mother trees and their progeny, but we mostly found no evidence of significant among-families genetic variation (except for density of axial ducts) (Fabián-Plesníková et al., 2021). We did not previously explore, however, genetic variation in traumatic resin ducts, despite the relevance of this trait. In this work we hypothesized that if traumatic duct traits are highly heritable at family level, their association with resin yield would be a central issue, in order to eventually focus the selection process on the traumatic ducts and achieve an increase in the resin yield of *Pinus oocarpa* trees. The decision of on what trait need to be focused the selection process on a breeding program, is a major decision, and difficult to take when according to plant defense theory (Coley, 1985; Herms and Matson, 1992), growth and defenses may trade-off; thus, negative associations between resin flow and growth may also be expected, as has been recently observed in other highly tapped pine species (Zas et al., 2020a; Vázquez González et al., 2021).

Thus, understanding the genetic variation in growth traits, resin duct anatomy, and resin yield among families is a first step in exploring appropriate strategies for a breeding program aiming to improve resin production in *Pinus oocarpa*. Also, research on the anatomy of traumatic ducts and its relation to resin yield could help to understand the potential productivity of the species (Rodríguez-García et al., 2014; Neis et al., 2019b). A high correlation between some of the assessed traits with resin yield, could mean that a higher number of high-yielding trees could be selected in less time. Also, that an early evaluation system could be established to predict future behavior with enough advance and increase of the genetic gain per unit of time (Wu, 1999).

Therefore, this study (i) explores the genetic variation among families in growth, traumatic resin ducts (formed after the wounding by microchipping), and resin yield in *P. oocarpa* and estimates heritability values and genetic gains, and (ii) explores the association between growth traits and resin yield and between resin ducts and resin yield.

Materials and methods

Plant material

The study material consisted of 30 open-pollinated families on a progeny field tests and their mother trees at natural stands. All plus trees are in the Ejido San José de Cañas, Ario´s Municipality, state of Michoacán de Ocampo, central-west Mexico, in an area of approximately 1,007 ha (Figure 1). The selected mother-trees are in the property of local resin tappers and were considered by them as high-yielding trees. Selected mother-tree characteristics were described in detail in Reyes-Ramos et al. (2019). Seeds were collected from those trees; seedlings were grown in a forest nursery and a progeny

test was established in 2011. The progeny tests field experimental design was a randomized complete block design, with 10 blocks, 3 seedlings per discontinuous plot, and 3 x 3 m spacing. Because of the mortality of some seedlings, 24 families were finally retained in the progeny test and for the statistical analysis. All the alive trees were measured on the progeny test for growth traits and resin production (average of 16 trees per family) and only a subset for anatomical resin duct traits (more details below).

Selection and collection of samples for anatomical studies

For the analyses here presented we chose a subset of four high yielding families and four low yielding families from the field progeny tests, that were identified as so by previous resin yield assessments. From those chosen families, they were sampled an average of nine trees per family, each individual from different blocks. Anatomical samples were obtained by removing the wood sample 10 cm above the wound done during the microchipping, which was made 20 cm above the soil surface.

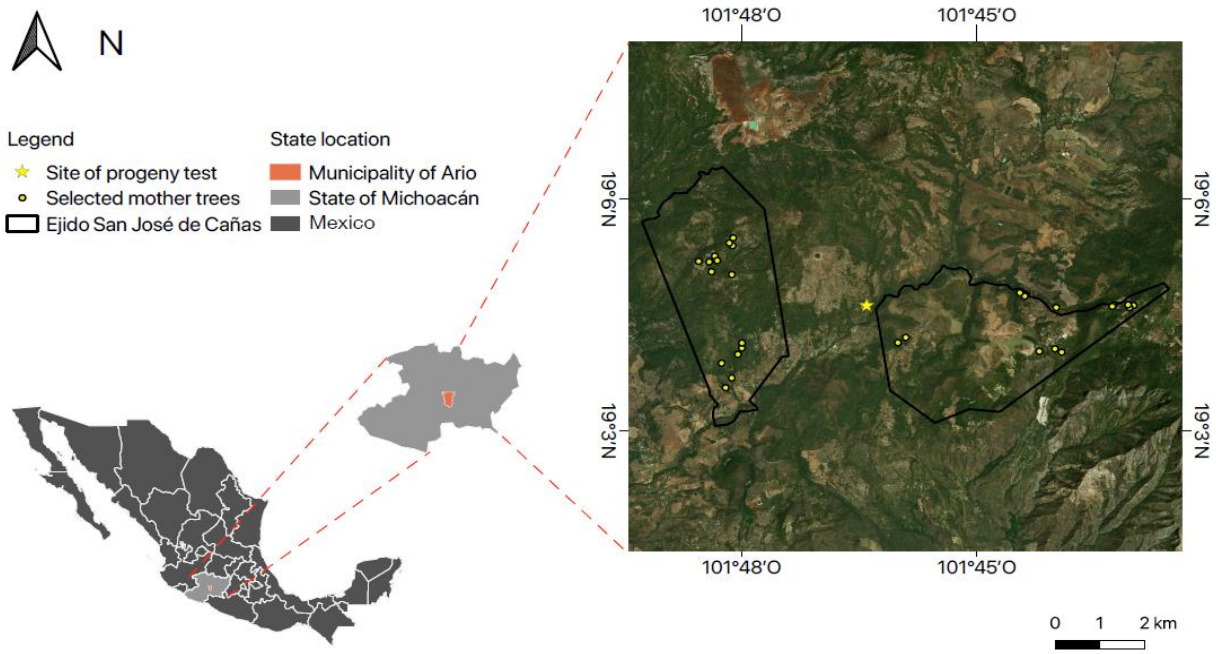


Figure 1
Location of the field progeny trial (yellow star) and mother-trees selected as phenotypically superior in resin yield (yellow circles) of *Pinus oocarpa* in the municipality of Ario, state Michoacán, Mexico.

Tree size measurements

We measured total tree height (HT) with an adjustable extension, and basal diameter (BD) and breast height diameter (DBH) with a caliper. Stem volume (VOL) over bark was calculated at age five from the HT and diameter measurements using the volume formula by Smalian cited by Riaño-Melo and Lizarazo (2017):

$$VOL\ (m^3) = \frac{1\pi}{4}\left(\frac{D_1^2 + D_2^2}{2}\right)L$$

where π is 3.1416, D_1 is the large-end diameter (cm^2), D_2 is the small-end diameter (cm^2), and L is the length (cm). More details of diameter and tree height measurements of progeny trees, as well as results description, were presented elsewhere (Fabián-Plesníková et al., 2020). Here we focus on the genetic variation of these growth traits at 5-years-old and its relationship with resin yield (at 6-years-old) and with anatomical traits.

We sampled additional individuals from the rows of trees planted for controlling the border effect in the field progeny tests, which were never tapped before; that were collected only to have a control lot to be used for estimation of genetic gains. Wood samples were extracted with a metal punch, stored in formalin-acetic acid-ethanol (FAA), and then prepared for light microscopy observation using standard techniques (Ruzin, 1999). Serial sections (20-30 µm thick) were dehydrated (with ethanol), stained (with safranin-Fast-green) (Ruzin, 1999), and mounted permanently in synthetic resin. Half of the sections were cleared with 3 % commercial clorox bleach (to wash out the dark contents). Microphotographs were used to measure the resin duct traits. We measured the area and the diameter (also called "Feret" diameter) of the traumatic ducts (TD) with the software Fiji with the elipse tool, ImageJ Version: 2: 0.0-rc-69/1.52i (Schindelin et al., 2012).

In order to differentiate traumatic resin ducts from constitutive resin ducts, we adopted the criteria that TDs generally form a tangential band (Panshin and de Zeeuw, 1980;

Vázquez-González et al., 2020; López-Villamor et al., 2021). That means that more than two canals are side by side, forming a protective barrier. Thus, all the traumatic ducts reported were those forming tangential bands of more than two canals in a row. More details of sampling, laboratory processing, and anatomical descriptions are reported elsewhere (Fabián-Plesníková et al., 2021).

Resin yield assessment

We quantified the resin yield capacity of the families established in the progeny trial by the experimental method called microchipping. Microchipping or microtapping consists of making small incisions in the tree to estimate resin yield at an experimental level (Squillace and Gansel, 1968). The microchipping technique used on the progenies allowed us to shorten the assessment period of the selected families (Gurgel-Garrido et al., 1999; Zas et al., 2020a), and to evaluate resin yield at an early age on trees with small diameters (Squillace and Gansel, 1968) without causing significant damage.

First, we removed the bark and made an incision in the tree with a drill of approximately 8 mm in diameter at 20 cm from the soil surface. Then we applied 50 % sulfuric acid immediately after performing the wound with a syringe (Schopmeyer et al., 1954; Oliveira, 1987). The acid worked as a stimulant to obtain the maximum yield and increase the duration of the flow (Clements, 1974; Parham, 1976; Vázquez-González et al., 2021). We placed a slightly bent metal sheet below the wound so that the resin could flow directly into a pre-weighed, 50 ml, polyethylene container that was attached to the tree. Containers were collected and weighed seven days after that the experiment was established. The test was assessed on the progenies at (after field planting) three, four, five, and six years old, in April-May, when the harvesting season for resin is at its highest peak. Here, we will focus on the six-year-old resin yield only, in order to have a yield on the latest age measured.

In order to explore possible correlation for resin yield between mother trees and progenies, the resin yield of the mother trees was also harvested, although by an adaptation of the borehole technique (Lekha and Sharma, 2005), and the crude resin was harvested for 48 hours in 2017, the same year of the microchipping resin extraction for the progeny tests. The tree was wounded with a drill (0.5 inches in diameter) at three different points of the tree stem at the same height (approximately at breast height) and above the existing faces reaching a depth of 5 to 10 cm. Then we applied sulfuric acid to each wound with a 5 ml syringe and attached to the shaft of the tree a specialized sampler with a plastic bag secured with plastic screws to collect the resin sample and to protect it from environmental contamination (Karsky et al., 2004). After 48 hours, the resin was collected and weighed using a digital scale.

Statistical analysis

To explore genetic variation among families, we performed analyses of variance (ANOVA), using the GLM procedure of SAS, obtaining the variance components through the REML method (SAS, 2019). The following linear model was used for the analysis of resin yield and stem volume in the progeny trial:



Figure 2
Resin yield and anatomical assessment of *Pinus oocarpa*: (a) Evaluation of mother trees. (b) Evaluation of progenies by microchipping. (c) Macroscopic wood sample of an adult tree with distinctive axial and traumatic resin ducts (d) Traumatic ducts in cross-section. Scale bar is 2 mm in (c); 100 µm (d). a = axial ducts, t = traumatic ducts.

$$Y_{ijk} = \mu + \beta_i + \varphi_j + \beta\varphi_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the observed value of the k^{th} plant of the j^{th} family in the i^{th} block, μ is the overall mean of the experiment, βi is the effect of the i^{th} block, φ_j is the random effect of the j^{th} family [E (φ_j)= 0; Var $(\varphi_j)= \sigma^2_f$], $\beta\varphi_{ij}$ is the random effect of interaction between the i^{th} block and the j^{th} family [E $(\beta\varphi_{ij})$= 0; Var $(\beta\varphi_{ij})= \sigma^2_{bf}$], ε_{ijk} is the experimental random error [E (ε_{ijk})= 0; Var $(\varepsilon_{ijk})= \sigma^2_e$]. For the analysis of the subset of the anatomical traits (traumatic ducts and total area), the following model was used: $Y_{ijk} = \mu + \varphi_j + \varepsilon_{ijk}$. Due to the fragility of the tissue for the anatomical analysis, the number of individuals per family considered in the statistical analyses was variable. All the analyses were performed with SAS University Edition software version 9.4.

The heritability coefficients at individual plants level, average among families, and within families, were estimated using the following expressions (Zobel and Talbert, 1992):

$$h_i^2 = \frac{3\sigma_f^2}{\sigma_f^2 + \sigma_{bf}^2 + \sigma_e^2}$$

$$h_f^2 = \frac{\sigma_f^2}{\sigma_f^2 + (\sigma_{bf}^2/b) + (\sigma_e^2/nb)}$$

where σ^2_f is the family variance (additive), σ^2_{bf} is the block variance per family, σ^2_e is the residual variance, b is the number of blocks, and n is the harmonized average number of trees per family in each block. The coefficient of family variance used a

value of "3" instead of "4" to consider the possibility that some individuals actually would be full-sibs. The standard error of the heritabilities *SE* (h^2) was estimated by the adapted formula Jayaraman (1999):

$$SE\ (h_i^2) = \frac{\left(1-\frac{h^2}{3}\right)[1+nb-1]\left(\frac{h^2}{3}\right)}{\left[\left(\frac{nb}{2}\right)(nb-1)(f-1)\right]^{0.5}}$$

$$SE\ \left(h_f^2\right) = \frac{(1-t)(1+nbt)}{\left[\frac{(nb)(f-1)}{2}\right]^{0.5}}$$

There *t* is the intraclass correlation, which equals one-third of the single tree heritability, *n* is the harmonic mean of the individuals, *b* is the number of blocks in the trial, and *f* is the number of families in the progeny trial.

For the anatomical traits, reduced formulae of heritability were used, because we did not sample all the individuals from all the blocks from the chosen eight families.

$$h_i^2 = \frac{3\sigma_f^2}{\sigma_f^2 + \sigma_e^2}$$

$$h_f^2 = \frac{\sigma_f^2}{\sigma_f^2 + \frac{\sigma_e^2}{n}}$$

The genetic gain was estimated by the following equation (Diao et al., 2016):

$$\Delta\ G(\%) = \frac{i\ h_i^2\sqrt{\sigma_P^2}}{\bar{X}}\ x\ 100\%$$

where *i* symbolizes selection intensity, h^2_i the narrow-sense heritability, σ_p^2 the total phenotypical variance, and $\bar{X}$ the mean of the population.

We estimated Pearson correlations between averages per family of tree growth, anatomical traits of the TD, and resin yield, as a proxy of genetic correlations. Linear regressions were measured to assess the relationships between variables to explore if resin yield can be predicted based on any of the analyzed traits. The traits considered in the estimation of the possible relations between variables are detailed in Table 1.

Table 1
Descriptions of traits considered for the correlation analyses in this study and their abbreviations.

Trait	Description	Abbreviation	Unit
Growth traits*	Total height	HT	m
	Basal diameter	BD	cm
	Diameter at breast height	DBH	cm
	Stem volume	VOL	m^3
Anatomical traits**	Diameter traumatic ducts	TD diam	mm
	Area traumatic ducts	TD area	mm^2
Resin yield	Resin of progenies	.	g/tree
	Resin of mother trees	.	g/tree

*Growth traits were assessed at 5-years-old.
**Anatomical samples and resin yield were taken after the last microchipping at age 6 for the open-pollinated progenies.

Results

Genetic variation among families

We found significant genetic variation among families ($p \leq 0.05$) for all the assessed traits (growth, TDs, and resin yield) (Table 2). However, the contribution of the family variance to the total variance as percent was relatively low for growth traits: 4, 7, 5, and 3 %, for HT, BD, DBH, and VOL, respectively (Table 2), although such contribution indeed was statistically significant among families. In contrast, the largest genetic difference among families, expressed as a percent of contribution to total variance by the family as a source of variation, was by far for the TD traits: $\sigma^2_{f\%}$= 21 % for TD diam, and $\sigma^2_{f\%}$= 50 % for TD area. Such large genetic variation is also expressed as the highest values of heritability of the TD traits: h^2_f= 0.51 and h^2_f= 0.81, respectively (Table 2). The high heritability on an individual basis for TD area (h^2_i= 1.60), was out of the expected maximum value theoretically possible (1.00). Resin yield had a moderate to low amount of genetic variation among families, with a 7 % contribution to total variance by the family source of variation, with a family heritability of h^2_f= 0.52. Resin production of mother trees had a large contribution to total variance by the mother trees (34 %).

Average family performance and expected genetic gains

When sorting the performance of resin yield, either for family average in the progeny tests or of the mother trees, it is evident that some families are higher resin yielders, as well as their mother trees, and that have also large (in diameter and area) TD. Those are for example the families coded as 16 and 20 (Supplementary Table 1; Figure 3).

Conducting a selection of the best individuals with an intensity selection of *i*= 1.4 would make an expected genetic gain for traumatic duct diameter and area of 13 % and 45 %, respectively (Table 2). If selecting for the progenies with the largest resin production (*i*= 1.4 also), the expected genetic gain is also large, 25 %.

Correlations among traits and genetic gains

When examining the relationship between resin production and growth traits, the correlation is weak. Phenotypic Pearson correlation (*r*) of progenies resin yield against tree height, basal diameter, diameter at breast height, and stem volume, were 0.17, 0.16, 0.17, and 0.18 ($p<0.05$), respectively. In contrast, linear regression values for resin yield against diameter and area of TD were higher r^2= 0.53 (p= 0.04) and r^2= 0.5 (p= 0.0479),

Table 2
Genetic parameters of a progeny trial of *Pinus oocarpa* in Mich., Mexico (half-sib progeny overall mean values, coefficients of variation, contribution to the total variance of each source of variation and its significance, heritability and expected genetic gains for growth traits (tree height, diameter, volume), traumatic ducts (diameter and area), resin yield (by microchipping for seven days), resin yield (48 hrs. extraction) of the mother trees.

				Variance components (%)								
Trait	*N**	*Mean*	*CV*	$\sigma^2_{f\%}$	*p*	$\sigma^2_{b\%}$	*p*	$\sigma^2_{bf\%}$	*p*	$\sigma^2_{e\%}$	h^2_i	h^2_f	$\Delta G_{\%}$
Height (m)	383	5.51	20	4	0.0478	17	<.0001	4	0.2518	75	0.15(0.03)	0.44(0.12)	4
Basal diameter (cm)	386	13.44	18	7	0.0023	9	<.0001	4	0.3887	80	0.22(0.03)	0.54(0.14)	5
Diameter at breast height (cm)	386	10.38	20	5	0.0181	9	0.0002	4	0.3526	82	0.17(0.03)	0.47(0.13)	5
Volume (m^3)	383	0.07	47	3	0.041	13	<.0001	9	0.2268	75	0.25(0.04)	0.46(0.16)	12
Traumatic ducts diameter (mm)	74	0.17	21	21	0.0536	.	.	.	.	79	0.63(0.10)	0.51(0.0.64)	13
Traumatic ducts area (mm^2)	74	0.013	42	50	0.0014	.	.	.	.	50	1.60(0.15)	0.81(0.57)	45
Resin progenies (g)	361	12.97	91	7	0.0002	0	0.3499	0	0.1348	93	0.20(0.04)	0.52(0.22)	25
				$\sigma^2_{tree\%}$									
Resin mother trees (g)	24	95.42	85	34	0.0026	.	.	.	.	66	.	.	.

Note: Trait overall mean (Mean); number of sampled trees (N) (* for growth traits and resin yield 24 families were included in the analyses; for anatomical traits 8 families); coefficient of variance, expressed as % (CV); p= significance of the ANOVA; percent of contribution to total variance by family variance ($\sigma^2_{f\%}$), block variance ($\sigma^2_{b\%}$), block*family variance ($\sigma^2_{bf\%}$), and error ($\sigma^2_{e\%}$); narrow sense heritability at individual (h^2_i) and family (h^2_f) level, with standard error in parenthesis, and expected genetic gains expressed as percent ($\Delta G\%$). For the resin yield of the mother trees, there is variance among trees (σ^2_{tree}), instead of among families.

respectively. For the mother trees in the field, a similar association between TD size and resin yield was obtained. The linear regression values for resin yield against diameter and area of TD were r^2= 0.69 (p= 0.011) and r^2=0.63 (p= 0.0193), respectively (Figure 3).

Discussion

Traits related to tree growth, like HT, BD, DBH, and VOL had low heritability values (when comparing with resin yield and traumatic duct traits), suggesting that those traits are under stronger environmental influence, and consequently under a relatively lower genetic control. The fact that such traits are poorly associated with resin yield is a result also found in other species, like *P. elliotii* (Gurgel-Garrido and Kageyama, 1993; Romanelli, 1995) and *P. caribaea* (dos-Santos et al., 2016), indicates that tree size (HT, diameter, and VOL) might not be a trait on which to conduct tree selection, in order to increase the resin production. Apparently, the selection of genotypes of larger growth rate, by selection based on HT, BD, DBH or VOL, might not be the best strategy given that low association between those growth traits and resin production. The widely accepted perception among resin tappers that larger trees might be better resin producers, could come from the fact that larger adult trees have larger diameters, which allows them to have a larger area of the stem tapped by traditional methods.

The higher degree of genetic variation among open-pollinated progenies for resin yield as opposed to stem volume, which showed a lower degree of variation, has been reported previously for *P. elliottii* (Lai et al., 2017). However, our results might be partially biased by the fact that the progenies tested were originated from mother trees identified in the field as high resin yielders, independently of their stem volume (Squillace and Bengtson, 1961).

The large contribution of family variance to the total variance, and the high heritability values for TD´s diameter (h^2_f= 0.51) and TD area (h^2_f= 0.81), suggest a strong genetic control at family level of the inducibility of TD with large diameter (and consequently in area) in response to the wounds inflicted by resin extraction. These values could be considered as high heritability, considering that <0.15 is low, 0.15 to 0.50 is moderated and >0.50 is high (Resende 2002; dos-Santos et al. 2016). The strong positive association between the TD diameter and the amount of resin production, indicates that possibly the individual trees that are high resin yielders are so, because of their capability to vigorously respond to a mechanical wounding by creating large (in diameter and area) TD, and that such capability is heritable. Eventually, selecting trees with higher resin production may also work as selecting resistant trees, since Zhao and Erbilgin (2019) have found that trees with larger but fewer constitutive resin duct had a greater probability of survival during and outbreak of mountain pine beetle attacks.

The strong association between size of the TD with resin production, confirms the importance of the contribution of the TD formation to the resin yield since such ducts and their associated cells constitute the site of production, and mobilization of resin to the injured or attacked site (Lombardero et al., 2000) in response to environmental disturbances (DeRose et al.,

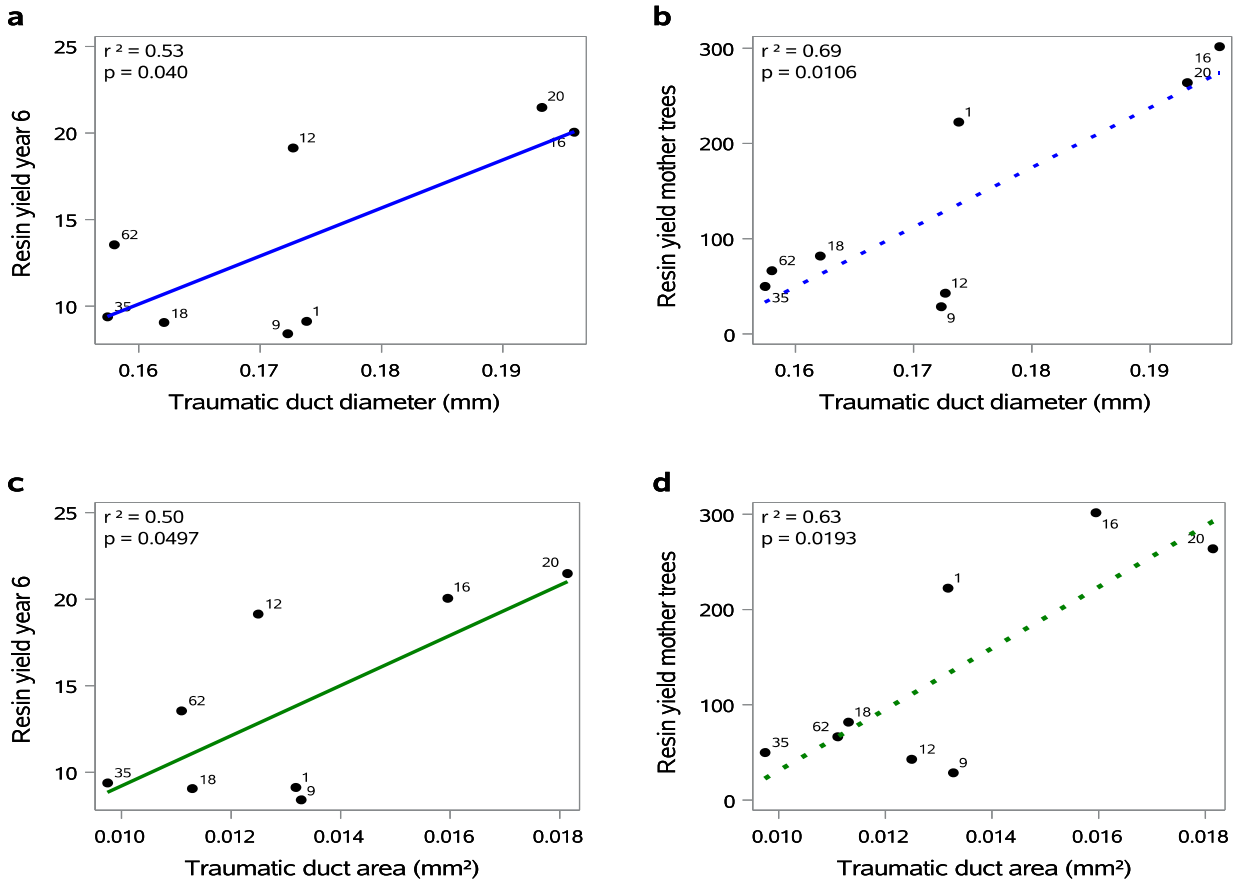


Figure 3
Linear regression of resin yield of the open-pollinated progenies (a, c) and of the mother trees (b, d), against diameter and area of traumatic ducts (in all panels) of the open-pollinated progenies. Family code is indicated beside the symbols. Fitted linear regression is indicated with lines (continuous line when fitted progenies' resin yield; dotted line when fitted mother trees' resin yield), as well as r^2 and significance of the regression.

2017). The high and positive correlations between resin production and the TD area could be explained by the Hagen–Poiseuille´s equation, which implies that even a small increase in diameter has an important impact on the conducting capacity of the ducts (Schopmeyer et al., 1954; Sperry et al., 2006).

The estimation of a significant heritability of resin production in the progenies (h^2_f= 0.52) confirms its genetic control at family level. That result seems also confirmed by the significant variability for resin yield among mother trees ($\sigma^2_{families}$ = 38%), tapped in the natural forests. However, it is needed strongly to stress that the latter is not a genetic variance because the mother trees were not in an experimental setting: they were resin production assessed in the natural forest; thus, variation among mother trees is completely phenotypical: it is the result of their genotype and the influence of the microsite where the adult trees grew.

Altogether, our results indicate that a tree breeding program for increasing resin production could be moved forward either by an early selection of genotypes with the highest resin yield, assessing by microchipping or by selecting genotypes with larger diameter and area of TD (whose formation could be induced by microchipping), or a combination of both criteria in a selection index (to be constructed).

Since the narrow-sense heritability for resin production at family level was much higher than at individual level, the selection would be more efficient if conducted on a family basis, and then for best individual within selected families (Susilowati et al., 2013; Zeng et al., 2013; dos-Santos et al., 2016; Lai et al., 2017).

An example of very good candidates for selection would be families coded as 16 and 20 (See family ranking on Supplementary Table 1). Those families are evidently good candidates for selection to be included in a seed orchard whose priority would be to produce genetically improved seed of genotypes that could increase the resin production. The expected genetic gains, when selecting for resin yield (25 %) or traumatic duct area (45 %) would be very acceptable, and the former is in agreement with what has been obtained on *P. elliottii* (Wright, 2012).

Limitations of this work

Even though resin production in Mexico is one of the most economically important non-wood forest products, we do not know previous estimates of the genetic control of this relevant trait to compare our findings. However, the genetic control of resin production has been documented outside Mexico and for other species around the world, like *Pinus caribaea* var. *bahamensis*, *P. caribaea* var. *hondurensis, P. elliottii, P. massoniana, P. merkusii*, and *P. pinaster* (Gurgel-Garrido et al.,1996; Tadesse et al., 2001; Liu et al., 2013; Susilowati et al., 2013; Zeng et al., 2013; dos-Santos et al., 2016; Lai et al., 2017).

The results above the theoretical limit for heritability of the TDs might indicate that in the estimated value of variance among families, it might be a confounding effect. Possibly, it might be a no-heritable vigorous response for TD formation, as a response to the tapping wound, that makes overestimation of the among family variance. Also, a possible source of overestimation, is that it was estimated from a subset of families selected either for high or low resin production in the progeny test. However, a very high heritability in anatomical structures has been observed in the number of resin ducts per needle in *Pinus nigra* of Peloponnese (h^2=0.97; 0.77) (Matziris 1982; Matziris 1984).

A closer examination of the variation among early ages for resin production and the development of TD would be desirable, in order to find the earliest age at which it would be feasible to conduct selection with genetic gains that would be worth it. Other studies have shown a significant age-age correlation for resin yield (Squillace and Gansel, 1974; Gurgel-Garrido and Kageyama, 1993; Gurgel-Garrido et al., 1994; Romanelli 1995; Romanelli and Sebbenn, 2004; Zeng et al., 2013), suggesting an opportunity for early selection (Pswarayi et al., 1996; Romanelli and Sebben 2004). However, if ontogeny has relevant effects on resin flow, as it was found by Zas et al. (2020b), future work should examine if effectively high-resin yielding young pines continue to be so when becoming mature reproductive individuals.

Also, the present results are based on a single progeny test established at one site. It would be needed to replicate similar studies in contrasting environments in order to have an estimation of the amount of contribution of the environment to the total variance for resin production and traumatic duct formation.

Conclusions

The high heritability values found for resin production, for TD diameter and TD area, and the significant association between resin yield and traumatic duct size, suggest that those traits are under strong genetic control at open-pollinated family level. Thus, a tree breeding program for increasing resin yield in *P. oocarpa* is feasible selecting at early ages (six-years-old in our case) the best trees based on resin yield (using the microchipping technique for the assessment at such early age), and/or selecting trees with large diameter and area of TD (after been wounded for tapping).

Therefore, selecting superior trees for resin yield, and developing genetically improved seed orchards for resin production would facilitate the establishment of intensive plantations. That would be an alternative for both the conservation of *P. oocarpa* valuable genetic resources and for generating an economically profitable alternative in Mexico. That would be relevant in particular in the state of Michoacán, where the *P. oocarpa* natural stands are aggressively removed to establish avocado orchards for exportation.

Acknowledgments

We thank to Ph.D. Brian Strom, from the USDA Forest Service, for facilitating the resin samplers, to the Coordinación de la Investigación Científica of UMSNH to NMS-V for funding, and to the Mexican Council of Science and Technology (CONACyT) for a fellowship (414524) to IF-P. We are also grateful to Jesús Morales-Hernández for helping during fieldwork, and Ph.D. Alicia Rojas-Leal for supporting during sample processing at UNAM, and to all the resin tappers from the Ejido San José de Cañas, Michoacán, México for allowing to sample their trees. Comments by two anonymous reviewers significantly improved the manuscript.

References

Boschiero AT, Tomazzello-Filho M (2012) Anatomical aspects of resin canals and oleoresin production in pine trees. In: Fett-Neto AG, Rodrigues-Corrêa KCS (eds) Pine resin: biology, chemistry and applications. Kerala, India: Research Signpost, pp 9-24, ISBN: 978-81-308-0493-4

Cabrita P (2021) A model for resin flow. In: KG Ramawat, HM Ekiert, S Goyal (eds) Plant cell and tissue differentiation and secondary metabolites. Reference Series in Phytochemistry. Springer, Cham, pp 1-28. https://doi.org/10.1007/978-3-030-30185-9_5

Clements RW (1974) Manual: Modern gum naval stores methods. USDA Forestry Service General Technical Report, Southeast Forest Experiment Station, Asheville, North Carolina, pp 1-29

Coley PD (1988) Effects of plant growth rate and leaf lifetime on the amount and type of anti-herbivore defense. Oecologia 74(4):531-536. https://doi.org/10.1126/science.230.4728.895

Coppen JJ, Hone GA (1995) Non-wood forest products, vol. 2. Natural Resources Institute, Food and agriculture organization of the United Nations (FAO), Rome, Italy

da Silva Rodrigues-Corrêa KC, de Lima JC, Fett-Neto AG (2013) Oleoresins from pine: production and industrial uses. In: Ramawat K., JM Mérillon (eds) Natural Products. Berlin, Germany: Springer, 4038-4051. https://doi.org/10.1007/978-3-642-22144-6_175

DeRose RJ, Bekker MF, Long JN (2017) Traumatic resin ducts as indicators of bark beetle outbreaks. Canadian Journal of Forest Research 47(9):1168-1174. https://doi.org/10.1139/cjfr-2017-0097

Diao S, Hou Y, Xie Y, Sun X (2016) Age trends of genetic parameters, early selection and family by site interactions for growth traits in *Larix kaempferi* open-pollinated families. BMC Genetics 17(1):104. https://doi.org/10.1186/s12863-016-0400-7

dos-Santos W, Souza DCL, de-Moraes MLT, de-Aguiar AV (2016) Genetic variation of wood and resin production in *Pinus caribaea* var. hondurensis Barret & Golfari. Silvae Genetica 65(1):31-37. https://doi.org/10.1515/sg-2016-0004

Dvorak WS, Potter KM, Hipkins VD, Hodge GR (2009) Genetic diversity and gene exchange in *Pinus oocarpa*, a Mesoamerican pine with resistance to the pitch canker fungus (*Fusarium circinatum*). International Journal of Plant Sciences 170(5):609-626. https://doi.org/10.1086/597780

Fabián-Plesníková I, Sáenz-Romero C, Cruz-de León J, Martínez-Trujillo M, Sánchez-Vargas NM (2020) Parámetros genéticos de las características de crecimiento de un ensayo de progenies de *P. oocarpa*. Madera y Bosques. 26(3):1-14. https://doi.org/10.21829/myb.2020.2632014

Fabián-Plesníková I, Sáenz-Romero C, Cruz-de León J, Martínez-Trujillo M, Sánchez-Vargas NM, Terrazas T (2021) Heritability and characteristics of resin ducts in *Pinus oocarpa* stems in Michoacán, México. IAWA Journal 42(3) :258-278. https://doi.org/10.1163/22941932-bja10055

Franceschi VR, Krokene P, Christiansen E, Krekling T (2005) Anatomical and chemical defenses of conifer bark against bark beetles and other pests. New Phytologist 167(2):353-376. https://doi.org/10.1111/j.1469-8137.2005.01436.x

Francisco-Arriaga F, García-Rojas G, Hilda R, Kido-Cruz A, Cortés-Zavala MT (2011) Ingreso generado por la recolección de recursos forestale en Pichátaro, Michoacán, México. Agricultura, Sociedad y Desarrollo 8(1):107-117.

Gurgel-Garrido LM, Kageyama PY (1993) Evolução, com a idade, de parâmetros genéticos de *Pinus elliottii* var. elliottii Engelm., selecionado para a produção de resina. Revista do Instituto Florestal de São Paulo 5(1):21-37

Gurgel-Garrido LM do A, Ribas C, de O Garrido MA (1994) Variabilidade da produção de resina em *Pinus elliotti* Engelm. var. elliottii. Revista Instituto Florestal São Paulo 6:113-128.

Gurgel-Garrido LM do A, de O Garrido MA, da S Pires CL, Palomo M (1999) Variacão genética em progênies e procedências de Pinus caribaea Mor. var. bahamensis Barr. et Golf. para producão de resina e características de crescimento. Revista Instituto Florestal 11(2):105-121.

Herms DA, Mattson WJ (1992) The dilema of plants: to grow or defend. The Quarterly Review of Biology 67(3):283-335. https://doi.org/10.1086/417659

Jayaraman K (1999) A statistical manual for forest research. Forestry research support programme for Asia and the Pacific. Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific (FAO). Available at http://www.fao.org/3/x6831e/x6831e13.htm

Karsky D, Strom B, Thistle H (2004) An improved method for collecting and monitoring pine oleoresin. USDA Forest Service, Missoula Technology and Development Center, Missoula, Montana, USA, pp 1-4.

Krokene P (2016) Carbon castles and insect invaders: dissecting physical defences in conifer stems. Plant, Cell and Environment 39(8):1643-1645. https://doi.org/10.1111/pce.12687

Lai M, Dong L, Yi M, Sun S, Zhang Y, Fu L, Xu Z, Lei L, Leng C, Zhang L (2017) Genetic variation, heritability and genotype × environment interactions of resin yield, growth traits and morphologic traits for *Pinus elliottii* at three progeny trials. Forests 8(11), 409. https://doi.org/10.3390/f8110409

Lai M, Zhang L, Lei L, Liu S, Jia T, Yi M (2020) Inheritance of resin yield and main resin components in *Pinus elliottii* Engelm. at three locations in southern China. Industrial Crops and Products 144:112065. https://doi.org/10.1016/j.indcrop.2019.112065

Lara-Chavez A, Flinn BS, Egertsdotter U (2011) Initiation of somatic embryogenesis from immature zygotic embryos of Oocarpa pine (*Pinus oocarpa* var. oocarpa Schiede). Tree Physiology 31(5):539-554. https://doi.org/10.1093/treephys/tpr040

Lekha C, Sharma K (2005) Borehole method of oleoresin tapping Chir pine. Forest Chemicals Review 115(3):11-17

Lewinsohn E, Gijzen M, Savage TJ, Croteau R (1991) Defense mechanisms of conifers: relationship of monoterpene cyclase activity to anatomical specialization and oleoresin monoterpene content. Plant Physiology 96(1):38-43. https://doi.org/10.1104/pp.96.1.38

Lin J, Hu Y, He X, Ceulemans R (2002) Systematic survey of resin canals in Pinaceae. Belgian Journal of Botany 135 (1-2):3-14.

Liu Q, Zhou Z, Fan H, Liu Y (2013) Genetic variation and correlation among resin yield, growth, and morphologic traits of *Pinus massoniana*. Silvae Genetica 62(1-2):38–43. https://doi.org/10.1515/sg-2013-0005

Lombardero MJ, Ayres MP, Lorio Jr P, Ruel JJ (2000) Environmental effects on constitutive and inducible resin defences of *Pinus taeda*. Ecology Letters 3:329–339. https://doi.org/10.1046/j.1461-0248.2000.00163.x

López-Upton J, Ramírez-Herrera C, Jasso-Mata J, Jiménez-Casas M, Aguilera-Rodríguez M, Sánchez-Velázquez J, Rodríguez-Trejo DA (2011) Situación de los recursos genéticos forestales en México. Food and agriculture organization of the United Nations (FAO): México City, Mexico

López-Villamor A, Zas R, Pérez A, Cáceres Y, Nunes-da Silva M, Vasconcelos M, Vázquez-González C, Sampedro L, Solla A (2021) Traumatic resin ducts induced by methyl jasmonate in Pinus spp. Trees 35(2):557-567. https://doi.org/10.1007/s00468-020-02057-9

Matziris D (1982) Genetic variation in number of resin canals per needle of the Black Pine of Peloponnese. Dasiki Erevna (Greece) 3(2):153-175

Matziris D (1984) Genetic variation in morphological and anatomical needle characteristics in the black pine of Peloponnesos. Silvae Genetica 33(4-5):164-169

Moreira X, Mooney KA, Rasmann S, Petry WK, Carrillo-Gavilán A, Zas R, Sampedro L (2014) Trade-offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences. Ecology Letters 17(5):537-546. https://doi.org/10.1111/ele.12253

Neis FA, de Costa F, de Araujo AT Jr, Fett JP, Fett-Neto AG (2019a) Multiple industrial uses of non-wood pine products. Industrial Crops and Products 130:248-258. https://doi.org/10.1016/j.indcrop.2018.12.088

Neis FA, de Costa F, de Almeida MR, Colling LC, de O Junkes CF, Fett JP, Fett-Neto AG (2019b) Resin exudation profile, chemical composition, and secretory canal characterization in contrasting yield phenotypes of *Pinus elliottii* Engelm. Industrial Crops and Products, 132:76–83. https://doi.org/10.1016/j.indcrop.2019.02.013

Oliveira JG de (1987) Avaliação da produção de resina em progênies de *Pinus elliottii* em idade juvenil. Master´s thesis, Universidade Estadual do Paraná.

Panshin AJ, de Zeeuw C (1980) Textbook of wood technology: Structure, identification, properties, and uses of the commercial woods of the United States and Canada. 4th edn. New York, USA:McGraw-Hill Book Company.

Parham MR (1976) Stimulation of oleoresin yield in conifers. Outlook on Agriculture 9(2):76-81. https://doi.org/10.1177/003072707600900207

Pearce RB (1996) Antimicrobial defences in the wood of living trees. New Phytologist 132(2):203-233. https://doi.org/10.1111/j.1469-8137.1996.tb01842.x

Pswarayi IZ, Barnes RD, Birks JS, Kanowski PJ (1996) Genetic parameter estimates for production and quality traits of *Pinus elliottii* var. elliottii in Zimbabwe. Silvae Genetica 45(4):216-222.

Resende MDV de (2002) Genética biométrica e estatística no melhoramento de plantas perenes. Brasilia, Brasil: Embrapa Informação Tecnológica. Colombo: Embrapa Florestas, p 975, ISBN 8573831618

Reyes-Ramos A, Cruz -de León J, Martínez-Palacios A, Lobit PCM, Ambríz-Parra JE, Sánchez-Vargas NM (2019) Caracteres ecológicos y dendrométricos que influyen en la producción de resina en *Pinus oocarpa* de Michoacán, México. Madera y Bosques 25(1):1-13. https://doi.org/10.21829/myb.2019.2511414

Riaño-Melo O, Lizarazo I (2017) Estimación del volumen de madera en árboles mediante polinomio único de ahusamiento. Colombia Forestal 20(1):51–62. https://doi.org/10.14483/udistrital.jour.colomb.for.2017.1.a05

Roberds JH, Strom BL, Hain FP, Gwaze DP, McKeand SE, Lott LH (2003). Estimates of genetic parameters for oleoresin and growth traits in juvenile loblolly pine. Canadian Journal of Forest Research 33(12):2469-2476. https://doi.org/10.1139/x03-186

Rodríguez-García A, López R, Martín JA, Pinillos F, Gil L (2014) Resin yield in *Pinus pinaster* is related to tree dendrometry, stand density and tapping-induced systemic changes in xylem anatomy. Forest Ecology and Management 313:47–54. https://doi.org/10.1016/j.foreco.2013.10.038

Romanelli RC (1995) Seleçao precoce em progenies de *Pinus elliotti* var. elliottii Engelm. Revista do Instituto Florestal, São Paulo 7(1):101-103

Romanelli RC, Sebben AM (2004) Parâmetros genéticos e ganhos na seleção para produção de resina em *Pinus elliottii* var. elliottii, no sul do Estado de São Paulo. Revista do Instituto Florestal, São Paulo 16(1):11-23

Ruel JJ, Ayres MP, Lorio PL Jr (1998) Loblolly pine responds to mechanical wounding with increased resin flow. Canadian Journal of Forest Research 28(4):596-602. https://doi.org/10.1139/x98-030

Ruzin SE (1999) Plant microtechnique and microscopy. Oxford, UK:Oxford University Press, 322 p. https://doi.org/10.1006/anbo.2000.1231

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V,

Eliceiri K, Tomancak P, Cardona A (2012) Fiji: an open-source platform for biological-image analysis. Nature Methods 9(7):676-682. https://doi.org/10.1038/nmeth.2019

Schopmeyer CS, Mergen F, Evans TC (1954) Applicability of Poiseuille's law to exudation of oleoresin from wounds on slash pine. Plant Physiology 29(1):82-87. https://doi.org/10.1104/pp.29.1.82

Sperry JS, Hacke UG, Pittermann J (2006) Size and function in conifer tracheids and angiosperm vessels. American Journal of Botany 93(10):1490-1500. https://doi.org/10.3732/ajb.93.10.1490

Squillace AE, Bengtson GW (1961) Inheritance of gum yield and other characteristics of slash pine. Proceedings Southern Forest Tree Improvement Conference 6:85-96

Squillace AE, Gansel CR (1968) Assessing the Potential Oleoresin Yields of Slash Pine Progenies at Juvenile Ages. U. S. Department of Agriculture - Forest Service 4

Squillace AE, Gansel (1974) Juvenile: mature correlations in slash pine. Forest Science 20(3):225-229

Susilowati A, Siregar IZ, Supriyanto, Wahyudi I, Corryanti (2013) Genetic variation, heritability and correlation between resin production character of *Pinus merkusii* high resin yielder (HRy) in Cijambu seedling seed orchard (SSO). Biotropia 20:122-133. https://doi.org/10.11598/btb.2013.20.2.257

Tadesse W, Nanos N, Auñon FJ, Alía R, Gil L (2001) Evaluation of high resin yielders of *Pinus pinaster* Ait. Forest Genetetics 8(4):271–278

Vázquez-González C, Zas R, Erbilgin N, Ferrenberg S, Rozas V, Sampedro L (2020) Resin ducts as resistance traits in conifers: linking dendrochronology and resin-based defences. Tree Physiology 40(10):1313–1326. https://doi.org/10.1093/treephys/tpaa064

Vázquez-González C, López-Goldar X, Alía R, Bustingorri G, Lario FJ, Lema M, de la-Mata R, Sampedro L, Touza R, Zas R (2021) Genetic variation in resin yield and covariation with tree growth in maritime pine. Forest Ecology and Management 482:118843. https://doi.org/10.1016/j.foreco.2020.118843

Westbrook JW, Resende MFR, Munoz P, Walker AR, Wegrzyn JL, Nelson CD, Neale DB, Kirst M, Huber DA, Gezan SA, Peters GF, Davis JM (2013) Association genetics of oleoresin flow in loblolly pine: discovering genes and predicting phenotype for improved resistance to bark beetles and bioenergy potential. New Phytologist 199(1):89-100. https://doi.org/10.1111/nph.12240

Wright J (2012) Introduction to forest genetics. London, United Kingdom: Academic Press INC, 463 p, ISBN: 9780323148887

Wright JA, Osorio LF (1992) Results of provenance and family within provenance trials of Pinus tecunumanii in Colombia, South America. Forest Ecology and Management 55(1-4):107–116. https://doi.org/10.1016/0378-1127(92)90095-q

Wu HX (1999) Study of early selection in tree breeding. 2. Advantage through shortening the breeding cycle. Silvae Genetica 48(2):78–83

Zas R, Touza R, Sampedro L, Lario FJ, Bustingorri G, Lema M (2020a) Variation in resin flow among Maritime pine populations: Relationship with growth potential and climatic responses. Forest Ecology and Management 474:118351. https://doi.org/10.1016/j.foreco.2020.118351

Zas R, Quiroga R, Touza R, Vázquez-González C, Sampedro L, Lema M (2020b) Resin tapping potential of Atlantic maritime pine forests depends on tree age and timing of tapping. Industrial Crops and Products 157:112940. https://doi.org/10.1016/j.indcrop.2020.112940

Zeng LH, Zhang Q, He BX, Lian HM, Cai YL, Wang YS, Luo M (2013) Age trends in genetic parameters for growth and resin-yielding capacity in masson pine. Silvae Genetica 62(1-2):7-18. https://doi.org/10.1515/sg-2013-0002

Zerbe P, Bohlmann J (2014) Bioproducts, Biofuels, and Perfumes: Conifer terpene synthases and their potential for metabolic engineering. In: Jetter R. (eds) Phytochemicals – Biosynthesis, Function and Application. Recent Advances in Phytochemistry, vol 44. Springer, Cham. https://doi.org/10.1007/978-3-319-04045-5_5

Zhao S, Erbilgin N (2019) Larger resin ducts are linked to the survival of lodgepole pine trees during mountain pine beetle outbreak. Frontiers in Plant Science 10, 1459. https://doi.org/10.3389/fpls.2019.01459

Zobel B, Talbert J (1992) Técnicas de mejoramiento genético de árboles forestales. Mexico City, México: Limusa, 545 p, ISBN 9681828569