UNIVERSIDAD MICHOACANA
DE SAN NICOLÁS DE HIDALGO
Instituto de Investigaciones Agropecuarias y Forestales
Programa Institucional de Doctorado en Ciencias Biológicas

“Estudio de caracteres que intervienen en la producción de resina y su posible control genético en *Pinus oocarpa*”

TESIS

Para obtener el grado de
DOCTORA EN CIENCIAS BIOLÓGICAS
Opción: Ciencias Agropecuarias, Forestales y Ambientales

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MORELIA, MICH., AGOSTO 2020

DEDICATORIA

En memoria de mi abuela.

AGRADECIMIENTOS

A la vida por darme esta oportunidad…nunca me imaginé que algún día podría recorrer este maravilloso camino de la ciencia. Es un camino que disfruté enormemente y agradezco de todo corazón a quienes hicieron posible esta aventura.

A toda mi familia por su apoyo incondicional, su cariño y por no perder su fe en mí.

Agradezco a los Dres. Nahum M. Sánchez Vargas, Miguel Martínez Trujillo, José Cruz de León por su apoyo, sus observaciones y por el tiempo invertido en ser parte de mi comité. Un agradecimiento muy especial a la Dra. Teresa Terrazas por recibirme en su laboratorio, por enseñarme tanto, por su amabilidad y por hacer mi sueño realidad. También al Dr. Cuauhtémoc Sáenz Romero por estar allí en los momentos más difíciles, por su asesoría, paciencia y todos sus consejos. A la Dra. Alicia Rojas por todo lo que me enseñó en el laboratorio, su apoyo y amistad. Al Dr. Brian Strom, del Servicio Forestal de Estados Unidos (US Forest Service) por su valioso apoyo con los materiales para los experimentos de resinación.

Al profesor PhD. Arthur Fett-Neto por permitirme cumplir mi sueño de conocer las plantaciones resineras en Brasil, por acogerme en su laboratorio, por su amabilidad y su confianza. A mis amigos brasileños a cada uno de ellos agradezco por los momentos compartidos, por preocuparse por mí y por introducirme en su cultura. Gracias a Kelly (“my buddy”), Anita, Cibelle, Kelen, Johnatan (John), Nico, Betina, Yohanna, Cassia, Anna, Marry, Laura.

A mis compañeras de laboratorio Erika, Ana Laura, Ceci, Esme por su amistad. Mis agradecimientos van también a mis compañeros de la UNAM por su apoyo y amistad, a Eunice, Marisol, Dany, Alejandro, Emilio, Silvia, Gina y todos los compañeros del laboratorio de la Dra. Hilda (Alberto, Ceci, Mely, Pato, Alejandro, María, Angie)

A mis compañeros, Alejandro Reyes Ramos (por no dejar que pierda el ánimo y la esperanza), a Jesús Morales Hernández y Larita quienes me acompañaron desde principio a fin en la caza de pinos resineros y en todas las aventuras de campo. Sin su valioso apoyo este trabajo no hubiera sido posible.

Así mismo, quiero agradecer a quienes me apoyaron para superar la travesía de los trámites administrativos - Lilí y Mony. Al Dr. José Herrera Camacho y al Dr. Gerardo Rodríguez Alvarado por siempre estar allí y velar por nosotros.

Y, por último, pero no menos importante, a mis amigos los resineros del Ejido San José de Cañas, especialmente a Doña Evelia, Doña Rita, Don Toño y su papá, a Don Manuel, Javier Santillán, Don Miguel, Carrillo, Raymundo, Juanito y Javier, por dejarme asomar a este mundo tan noble y apasionante de la resina, estaré siempre agradecida.

Gracias a todos! Obrigada! Díky všem!

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RESUMEN GENERAL

Los bosques naturales son la fuente principal de extracción de resina en México, siendo *Pinus oocarpa* una de las especies más productoras de resina. El valor económico y ecológico de las poblaciones naturales actuales hace relevante revalorar la importancia de esta especie, frente a las condiciones adversas que enfrentan hoy en día los bosques a causa del cambio climático (sequías prolongadas y plagas más frecuentes y agresivas) y la deforestación por el cambio de uso de suelo. En México no existe un programa de mejoramiento genético de esta especie, que tenga como objetivo incrementar la producción de resina, quizás porque no se sabe mucho sobre cuáles serían los caracteres específicos por mejorar haciendo selección indirecta (la producción de resina comercial es un carácter que se expresa en una edad tardía) y son escasos los trabajos que han tratado de identificar y describir los caracteres que influyen en el proceso de resinación para la especie de nuestro interés en el país. Por lo anterior, el objetivo general del presente trabajo fue evaluar los caracteres de crecimiento, anatómicos, así como el volumen de producción de resina y su posible control genético en *Pinus oocarpa* Schiede ex Schltdl. Para ello se evaluaron las características de crecimiento (altura total, diámetro basal, diámetro a la altura del pecho) y se estimaron los parámetros genéticos (heredabilidad, correlaciones genéticas) en diferentes edades, en un ensayo de progenies establecido en el año 2011, en el Municipio de Ario, Michoacán, con 27 familias de medios hermanos. Se tomaron muestras de madera para la evaluación de los caracteres anatómicos (canales axiales y radiales) de 24 árboles madre y 74 individuos de ocho familias de medios hermanos (cuatro familias de producción alta, cuatro familias de producción baja). Las muestras se procesaron en laboratorio y se exploró su variación y posible control genético. Se evaluó la producción de resina de las progenies mediante la técnica de miniresinación y la producción de los árboles madre; se estimaron los parámetros genéticos para el volumen de resina, madera y los caracteres anatómicos tanto constitutivos, a través de un coeficiente (área total = media de área*número de canal), como los inducidos (canales traumáticos), así como las asociaciones entre los caracteres evaluados y la producción de resina. Los resultados sugieren la posibilidad de aumentar la media de la producción de resina y acortar los tiempos de evaluación de los genotipos a seleccionar. Además, un conocimiento más amplio sobre las características de la especie puede contribuir a generar alternativas para un mejor aprovechamiento futuro de los recursos forestales no maderables de la zona.

Palabras clave: canales resiníferos, ensayo de progenies, miniresinación, parámetros genéticos, selección temprana

SUMMARY

Natural forests are the primary source of resin extraction in Mexico, with *Pinus oocarpa* being one of the most resin yielding species in the country. The economic and ecological value of today's natural populations of *P. oocarpa* brings into attention the necessity of reassessing the importance of the species. Especially under the adverse circumstances that are facing forests nowadays due to climate change (more prolonged droughts and more frequent and aggressive pest attacks) and deforestation due to changes in land use. Breeding programs aimed to increase resin yield are absent in Mexico. Perhaps because not much is known about the specific characteristics that should be improved by an indirect selection (resin yield is a trait that is expressed at a late age), and there are only a few works that have tried to identify and describe the traits that influence the resin yield for the species of our interest. Therefore, the general objective of the present study was to evaluate the growth traits, anatomical characteristics, as well as the resin yield and its possible genetic control in *Pinus oocarpa* Schiede ex Schltdl. For that purpose, we assessed the growth traits (total height, basal diameter, breast height diameter) and we estimated genetic parameters (heritability and genetic correlations) at different ages in a progeny trial with 27 half-sib families, which was established in the municipality of Ario in the State of Michoacán, México. Wood samples for the assessment of anatomical traits (axial and radial ducts) were collected from the selected trees (24 mother trees) and 74 individuals from eight half-sibling families (four high-yielding families, four low-yielding families). All the samples were processed in the laboratory, photographed, and we explored the variation of the resin ducts and its possible genetic control. We assessed the resin yield of the 27 half-sib families by the microchipping technique and the resin yield of the mother trees and estimated genetic parameters for resin yield, stem volume and the anatomical traits both constitutive (estimated through a coefficient = total duct area = average area per duct * number of ducts) and induced (traumatic ducts), as well as the associations between the assessed traits with resin yield.

The results suggest the possibility of increasing the average volume of resin yield and of shortening of the assessment period of the selected genotypes. Moreover, detailed information about the species characteristics can provide better alternatives for the use of the non-timber forest resources in the area in the future.

Key words: resin ducts**,** progeny test, microchipping, genetic parameters, early selection

INTRODUCCIÓN GENERAL

La especie *Pinus oocarpa* tiene una amplia distribución en América Latina (Dvorak et al. 2000; Styles y Perry 1991). Su rango altitudinal óptimo se encuentra entre 1,200 a 1,800 m snm (Dvorak et al. 2000), pero se puede encontrar en un rango que va de 200 a 2,500 m snm (Cabi 2002). *P. oocarpa* fue identificada originalmente por el botánico de origen alemán, Christian Julius Schiede, en 1838 (Greaves 1979). Es una conífera de madera dura de la subdivisión Oocarpae (Gutiérrez-Vásquez et al. 2013), de 13 a 35 metros de altura, por 25 a 80 cm de diámetro, con la copa por lo general de forma redondeada (Dvorak et al. 2000).

Sus características naturales convierten a esta especie en una alternativa para la revegetación de áreas ociosas o deforestadas, ya que crece con relativamente facilidad en suelos degradados y su cultivo en vivero no es muy exigente (CABI 2002). La madera tiene una densidad de 0.45 – 0.60 g/cm^3 y se utiliza principalmente para trabajos de construcción, pisos, carpintería, durmientes de ferrocarril y postes (de transmisión, de cercas) (Webb et al. 1984). *P. oocarpa* ha sido introducido en varios países tropicales y subtropicales de África, Asia y América del Sur y en la actualidad se están llevando a cabo ensayos que estudian las propiedades de la madera de los híbridos de *P. oocarpa* con *P. patula*, *P. tecunumanii*, *P. caribaea* por la Cooperativa de Recursos de Coníferas de Centroamérica y México (CAMCORE) (CAMCORE 2019). En México, *P. oocarpa* es una fuente importante de extracción de resina, encontrándose entre las especies de mayor rendimiento (Gutiérrez-Jarquín 1977).

En México la resina se obtiene de manera comercial en bosques naturales a través del sistema de resinación francés o Hugues adaptado a México (Gutiérrez-Jarquín et al. 1979). Este método fue inventado en Francia y patentado en 1845 por el abogado, agricultor, e inventor, Pierre Hugues (Delgado-Macías 2015); es una técnica compatible con la vida del árbol y permite la extracción de resina durante varios años; su extracción está regulada por las especificaciones técnicas para la extracción de resina que se describen en la Ley General de Desarrollo Forestal Sustentable y su Reglamento, y la Norma Oficial Mexicana NOM-026-SEMARNAT-2005 (SEMARNAT 2006). Estudios recientes demuestran la existencia de individuos de *P. oocarpa* altamente productores de resina que sugieren la posibilidad de incrementar el potencial productivo de la especie (Reyes-Ramos et al. 2019). En otros países, para obtener resina a escala comercial, se ha inducido la producción de resina de manera artificial mediante diferentes tipos de sistemas de extracción (“chino”, “francés” o “de Hugues”, “americano”, “mazek o rill”) (Cunningham 2012); y en las últimas décadas se ha incrementado aún más la productividad de los árboles mediante la aplicación exógena de sustancias químicas, principalmente a base de ácido sulfúrico, que aumentan la producción y la duración del flujo de resina (da Silva Rodrigues-Corrêa et al. 2013).

Históricamente la resina ha sido considerada como el producto forestal no maderable de mayor importancia económica en México (Zamora-Martínez et al. 2001; Tapia-Tapia y Reyes-Chilpa 2008) y se recolecta principalmente en la región central del país en los estados de Michoacán, Jalisco, Estado de México y en sur en Oaxaca (Francisco-Arriaga 2011; Quiroz-Carranza y Magaña-Alejandro 2015). Actualmente, se reúnen en México aproximadamente 25,000 toneladas anuales (SEMARNAT 2017), posicionándose México en quinto lugar en el mundo (CONAFOR 2013), siendo países como China, Brasil e Indonesia los mayores productores (Baumassy 2019; Bai et al. 2020)

Además de su función ecológica, el hombre, ha aprovechado las cualidades de la resina para una gran diversidad de usos desde tiempos ancestrales: recientemente, se han encontrado indicios de que se utilizó durante la época neandertal como pegamento para herramientas (Degano et al. 2020); para los embalsamamientos de los fenicios, incas y cartagineses; los griegos y los romanos la ocuparon para iluminar, sellar y preservar barcos, edificios de madera y las urnas en ceremonias religiosas; para saborizar, purificar, preservar el vino y tratar las barricas (Meiggs 1982; Le-Maitre 2000). En las culturas prehispánicas la aprovecharon para alumbrar (los trozos de ramas con un extremo envuelto en fibras vegetales se impregnaban con resina de pino), obtener hollín (humo negro) para pintar, como pegamento (en las ceremonias se pegaban las plumas al cuerpo) o para sellar los recipientes donde se guardaba el pulque (Martínez-Cortés 1970). También tenía uso medicinal, y llegó a tener un papel tan importante que se creía que la Diosa Tzapotlatena, la diosa de los médicos y parteras había descubierto esta sustancia (Motte-Florac 2000). Los purépechas, mayas y aztecas usaban la resina en terapia contra el dolor de garganta, para el tratamiento de dislocaciones, fracturas y como antiséptico (Motte-Florac 1996).

Hoy en día los componentes de la resina tienen un importante uso industrial como precursores químicos por su gran diversidad estructural y múltiples propiedades químicas y físicas (Zerbe y Bohlmann 2014). Sus derivados, la brea y el aguarrás, se utilizan en una variedad de productos, como adhesivos, tintas, fragancias, agroquímicos, potenciadores de sabor, productos farmacéuticos (Bohlmann y Keeling 2008; da Silva Rodrigues-Corrêa et al. 2013; Neis et al. 2019a). Así mismo, se utilizan como componentes en la fabricación de baterías biodegradables, de plásticos ecológicos (Neis et al. 2019a) y potencialmente como precursores de biocombustibles especializados (Mewalal et al. 2017).

El aguarrás, es un líquido claro, inflamable. Su uso principal ha sido como disolvente y en la fabricación de pinturas, pero en la actualidad la aplicación más importante es la producción de materias primas para la fabricación de fragancias, aromas alimentarios, vitaminas, productos químicos y farmacéuticos, gomas y resinas sintéticas, pintura, barniz y laca, productos para ferrocarriles y astilleros, lustradores de calzado y materiales relacionados, hule, tintas de impresión, adhesivos y plásticos, productos asfálticos, muebles, insecticidas y desinfectantes (Ciesla 1998).

La brea es sólida, de aspecto vítreo, traslúcida y frágil, compuesta principalmente por ácidos abiéticos y pimáricos, insoluble en agua, pero soluble en solventes orgánicos (xilol, toluol, alcoholes) (Romahn-de la Vega 1982). Su color va del amarillo pálido al café rojizo (Romahn-de la Vega 1982). Sus usos principales están en la fabricación de tintas de impresión, como emulsionante en la fabricación de caucho sintético, materiales aislantes para la industria electrónica, eléctrica y térmica, revestimientos de superficies, linóleos, en adhesivos, como goma base para fabricar chicles, en jabones, detergentes, cosméticos, lacas, barnices y pinturas, insecticidas, germicidas, varios productos farmacéuticos y en la manufactura del papel (Coppen y Honne 1995; Ciesla 1998; Neis et al. 2019a).

La síntesis de la resina es principalmente un sistema complejo de defensa que, a algunas plantas, como los pinos, les ha permitido resistir los ataques de sus enemigos naturales (Martin y Croteau 1999), mediante la producción de una gran diversidad de metabolitos secundarios (Langenheim 1990). Estas defensas de naturaleza química o física residen en diferentes estructuras celulares y, por lo tanto, tienen una base anatómica (Krokene, 2016), cuyo propósito principal es proteger las funciones vitales de la corteza, del sistema vascular y la albura del árbol (Krokene 2016).

La mezcla de compuestos de la resina (monoterpenos y diterpenos en proporciones iguales y los sesquiterpenos en menor proporción) (Franceschi et al. 2005) y otro tipo de compuestos (Schmidt et al. 2005), resulta más difícil para los insectos para adaptarse que a un compuesto único (Langenheim 1994). Los monoterpenos y los sesquiterpenos son la parte volátil de la resina, actúa como un sistema de comunicación interno del árbol porque con un cambio en la concentración o composición emite una más eficiente alerta de ataque que si lo hiciera a través del sistema vascular, repele a insectos, hongos y microbios con una variedad de toxinas y otros agentes biológicamente activos y actúa como disolvente para transportar los ácidos resínicos de mayor peso molecular al sitio de la lesión (Gershenzon y Croteau 1991). Los ácidos resínicos (diterpenos) son la masa semicristalina que por su viscosidad atrapa y sofoca a los enemigos y formar una barrera endurecida que sella cualquier herida de forma inmediata para que no sufra una invasión microbiana o un daño mayor (Langenheim 1994; Martin y Bohlman 2005).

La resina se produce en las células epiteliales, que son células secretoras, parenquimatosas, de membranas delgadas no lignificadas (Fahn 1974); debido a su alta toxicidad se almacena bajo presión en conductos especializados llamados canales resiníferos, que son estructuras multicelulares rodeadas por capas de células epiteliales (Lewinsohn et al. 1991), su formación puede ser una característica normal del desarrollo en tejidos y órganos (canales constitutivos) o inducida por factores externos (canales traumáticos) (Pearce 1996). Se encuentran principalmente en los tallos, en la parte interna de la corteza (el floema secundario) y en la madera (el xilema secundario), pero se pueden encontrar también en las raíces, las hojas y los conos (Wu y Hu 1997). La resina constitutiva, actúa como una barrera mecánica (Krokene 2016), la cual inhibe o frena un ataque inicial; si esta acción defensiva no tiene el efecto esperado, las defensas inducibles se movilizan para matar o aislar a los enemigos e inicia la formación de los canales inducidos o traumáticos, conductos axiales en el xilema secundario (Nagy et al. 2000). La resina traumática, en particular, actúa como una barrera química, ya que parece tener diferente composición que la resina constitutiva (Nagy et al. 2000).

En este trabajo se explora la producción de resina en árboles seleccionados como buenos productores, el crecimiento de su progenie y su producción de resina, el papel que juegan algunos caracteres anatómicos de la madera en la mayor o menor producción de resina y la relación entre ellos, por esta razón el trabajo se ha dividido en tres partes: el Capítulo 1 trata sobre la evaluación del crecimiento de los árboles en un ensayo de progenies de *P. oocarpa* establecido en el Ejido San José de Cañas, Municipio de Ario en el año 2011, con 27 familias de medios hermanos, en un diseño de 10 bloques completos al azar; de donde se evaluó la variación y se obtuvieron parámetros genéticos tales como la heredabilidad y las correlaciones genéticas entre los caracteres medidos, y la ganancia genética esperada de los caracteres.

En el Capítulo 2 se describen los caracteres anatómicos que actúan en el mecanismo de defensa de la madera y la corteza de *P. oocarpa* para entender cómo los canales resiníferos varían entre los diferentes tejidos, edades y familias y se evalúa la magnitud de los parámetros genéticos; para lo que se analizan muestras de madera dos grupos de árboles, uno de 24 árboles-madre que mostraron ser superiores en producción de resina, con una edad promedio de 66 años y un diámetro a la altura del pecho de 62 cm; el otro de 74 individuos de un ensayo de progenie de seis años de edad procedente de los individuos superiores seleccionados mencionados en el Capítulo 1.

En el Capítulo 3, se evalúa el desarrollo temprano, heredabilidad y ganancia genética de los caracteres de crecimiento de los árboles (volumen de fuste) y producción de resina, y se relaciona con los caracteres anatómicos de la madera de una muestra de los árboles evaluados, para ello se utilizaron 24 árboles de *Pinus oocarpa* seleccionados por su alta producción de resina y 27 familias procedentes de esos árboles superiores; de los árboles se obtuvo la producción de resina por el método de miniresinación, y se midieron los caracteres anatómicos de la madera relacionados con la producción de resina.

JUSTIFICACIÓN

Los factores que influyen en la producción de resina van desde la fertilidad del suelo, el ambiente, la edad del árbol, la dimensión de los árboles, la sanidad de los árboles, la constitución genética, hasta los métodos de extracción (Ferreira 2009), por lo que se ha dificultado el estudio del proceso de resinación y en consecuencia el establecimiento de programas de mejoramiento genético enfocados a incrementar el volumen de producción de resina.

Por otro lado, cuando se busca correlacionar las estructuras morfológicas, anatómicas, así como las condiciones climáticas, con la producción de resina, los resultados de distintos estudios llevados a cabo en especies del género *Pinus* no parecen ser concluyentes y se reportan resultados diversos (Lai et al. 2017), quizá debido a la expresión fenotípica propia de cada una de las especies, por lo que no es posible generalizar estos resultados y es necesario llevar a cabo los estudios pertinentes en la especie *P. oocarpa*.

El mejor conocimiento de los caracteres y estructuras involucradas en la producción de resina y la exploración de la relación genética de los caracteres morfológicos, anatómicos y la producción de resina, podría reducir, en gran medida, los tiempos de selección. Así mismo, podría contribuir para formar una base científica para mejorar el aprovechamiento sustentable de los bosques en resinación.

La selección de un árbol superior en producción de resina hasta ahora se realiza en primera instancia a través de los productores (Reyes-Ramos et al. 2019). Si se pudiese encontrar una buena correlación entre algunos de los caracteres de los árboles madre con la producción de resina, se podrían seleccionar un mayor número de árboles en menor tiempo. De igual forma, la exploración de la relación genética de los caracteres morfométricos de las progenies y la producción de resina podría reducir, en gran medida, los tiempos de selección de los genotipos superiores.

HIPÓTESIS

Por lo anterior, las preguntas de investigación fueron: a) ¿Cómo se correlaciona (genotípica y fenotípicamente) el volumen de producción de resina con las características anatómicas (los canales resiníferos) y morfológicas (diámetro, altura y volumen) en árboles altamente productores de resina y sus progenies?; b) ¿Se pueden acortar los ciclos de selección de los genotipos superiores en producción de resina a través de la relación progenitor-progenie?

A su vez, las hipótesis del proyecto se especificaron de la siguiente manera: 1) Las características morfológicas (diámetro, altura, volumen), las anatómicas (densidad, tamaño y porcentaje de área de canales resiníferos) y la producción de resina varían entre familias de medios hermanos de *P. oocarpa* y están bajo control genético. 2) Los caracteres morfológicos, anatómicos y el volumen de producción de resina en progenies de *P. oocarpa* podrían estar relacionados y convertirse en un índice para la selección de genotipos superiores en producción de resina.

OBJETIVO GENERAL

Estudiar los caracteres de crecimiento y anatómicos de la madera, la manera en que influyen en la producción de la resina y evaluar su posible control genético en *Pinus oocarpa*.

OBJETIVOS ESPECÍFICOS

Los objetivos específicos del presente trabajo fueron:

- Evaluar el potencial de desarrollo de un ensayo de progenies de *P. oocarpa* seleccionado inicialmente por su alta producción de resina para considerar la mejora simultanea de resina y madera
- Identificar, describir y cuantificar, mediante técnicas de microscopía, los caracteres anatómicos implicados en la síntesis de la resina y su relación con el volumen de producción de resina.
- Estimar los parámetros genéticos (heredabilidad, correlaciones genéticas, regresiones) de las características anatómicas (tamaño y densidad de canales resiníferos, entre otros) y morfológicas (diámetro, altura y volumen) relacionadas con la producción de la resina.

PRODUCTOS GENERADOS

Los productos generados por esta tesis son los siguientes capítulos que están considerados para publicaciones en revistas científicas indexadas:

Capítulo 1: Fabián-Plesníková, Irenka; Sáenz-Romero, Cuauhtémoc; Cruz-de León, José; Martínez-Trujillo Miguel, Sánchez-Vargas, Nahum M. 2020. Parámetros genéticos de las características de crecimiento de un ensayo de progenies de *P. oocarpa*. Madera y Bosques (aceptado para publicación).

Capítulo 2: Fabián-Plesníková, Irenka; Sáenz-Romero, Cuauhtémoc; Cruz-de León, José; Martínez-Trujillo Miguel, Sánchez-Vargas, Nahum M.; Terrazas Teresa.

Capítulo 3: Fabián-Plesníková, Irenka; Reyes-Ramos Alejandro; Sáenz-Romero, Cuauhtémoc; Cruz-de León, José; Martínez-Trujillo Miguel; Terrazas, Teresa; Sánchez Vargas, Nahum M.

Adicionalmente, como parte del programa institucional de doctorado se publicó un artículo de portada en revista de divulgación indexada:

Fabián-Plesníková, Irenka; Terrazas Teresa. El arma química de los pinos. 2019. Saber más.

Capítulo 1 : Parámetros genéticos de características de crecimiento de un ensayo de progenies de *Pinus oocarpa*

Growth trait genetic parameters in a progeny trial of *Pinus oocarpa*

Resumen

Pinus oocarpa Schiede ex Schltdl. es una de las especies forestales en México de mayor importancia ecológica (por su amplia distribución geográfica) y económica (por la extracción de su resina en poblaciones naturales). Una resinación más intensiva requerirá de plantaciones comerciales, que podrían ser multipropósito: resina y madera. El objetivo del presente trabajo es conocer el control genético (heredabilidad) de caracteres de crecimiento (altura total, ALT), diámetro a la base del tallo (DIAM), y diámetro a la altura del pecho (DAP), de progenies de medios hermanos de árboles originalmente seleccionados para producción de resina. Esto con la finalidad de valorar la factibilidad de un programa de mejoramiento genético multipropósito. Se estableció un ensayo de progenies en el año 2011 en Michoacán, México con 27 familias de medios hermanos en un diseño de 10 bloques completos al azar. A los cinco años después de la plantación, los promedios fueron ALT = 5.5 m, DIAM = 13.4 cm y DAP = 10.4 cm. Hubo diferencias significativas entre familias para todos los caracteres. La heredabilidad a nivel individual (h^2_i) fue: ALT = 0.15, DIAM = 0.22 y DAP = 0.17; a nivel medias de familias (h^2_f) fue de 0.44, 0.54 y 0.47, respectivamente. A una intensidad de selección de mejores individuos de i = 1.4, las ganancias genéticas para ALT, DIAM y DAP fueron de 3.9, 5.2 y 5.0%, respectivamente. De demostrarse posteriormente asociación entre diámetro y producción de resina, sería factible incrementar ambos, mediante selección de individuos con mejores diámetros.

Palabras clave: correlaciones fenotípicas; correlaciones genéticas; crecimiento; ganancia genética; heredabilidad; intensidad de selección.

Abstract

In Mexico, *Pinus oocarpa* Schiede ex Schltdl. is one of the forest tree species of major ecological (for its wide geographical distribution), and economic importance (for the resin extraction in natural stands). More intensive exploitation will require establishing commercial plantations, which could be multipurpose: for resin tapping and wood. The present study aimed to explore the genetic control (heritability) of the growth characteristics for total height (HGT), stem base diameter (DIAM), diameter at breast height (DBH) of half-sib progenies from trees originally selected for its resin yield. All these in order to assess the feasibility of a multipurpose breeding program. In 2011, it was established a progeny trial in Michoacán, Mexico, with 27 open-pollinated half-sib families in an experimental design of 10 randomized complete blocks. Five years after its establishment, the mean values were HGT = 5.50 m, in DIAM of 13.29 cm, and DBH = 10.29 cm. We found statistically significant differences among families for all the characteristics. Narrow-sense heritability (h^2_i) was: HGT = 0.15, DIAM = 0.22, and DBH = 0.17; at family mean level (h^2_f) it was 0.44, 0.54, and 0.47, respectively. At an intensity of selection of the best individuals of i = 1.4, the genetic gains for HGT, DIAM, and DBH were 3.9, 5.2, and 5.0%, respectively. If, in the future, it is demonstrated that there is an association between diameter and resin yield, it would be feasible to increase both traits through the selection of the best individuals with the greatest diameters.

Keywords: phenotypic correlations; genetic correlations; genetic control; genetic gain; growth; heritability; selection intensity.

1.1 Introducción

Pinus oocarpa Schiede ex Schltdl. tiene amplia variación genética por la diversidad de ambientes en los que se encuentra de manera natural (Dvorak et al. 2000). Su distribución geográfica Norte-Sur comprende cerca de 3000 km, desde el noroeste y noreste de México hasta el Centro de Nicaragua (Dvorak et al. 2000). En México se encuentra en la Sierra Madre Oriental, Occidental y en la zona de transición sur (hacia la selva seca caducifolia) del Eje Neovolcánico (Gutiérrez-Vázquez et al. 2013). Es una de las especies con mayor variación altitudinal en México (en parte debido a su gran distribución Norte-Sur), que va desde los 200 m snm en el Noroeste de México hasta los 2500 m snm. Sin embargo, su óptimo se localiza entre 1200 m snm a 1800 m snm (Dvorak et al. 2000).

La madera de *P. oocarpa* proveniente de bosques naturales es de gran importancia como producto de exportación en varios países de América Central (especialmente en Honduras) (Dvorak et al. 2000). Si bien se han establecido plantaciones forestales comerciales con fines maderables, lo han sido como especie exótica (fuera de América Central y México; Dvorak et al. 2000), en zonas tropicales y subtropicales de Colombia, en la zona del Cerrado de Brasil y en varios países de África como Congo, Sudáfrica y Zambia (Gavidia 1978, Viveros-Viveros et al. 2005).

En México la madera de *P. oocarpa* se destina para aserrío, triplay, chapa, celulosa, papel, cajas de empaque, construcciones, duelas y en ebanistería (Zamora-Serrano 1981, Olvera-Coronel 1985, Quiroz-Carranza y Magaña-Alejandro 2015). Particularmente en zonas marginadas, es amplio su uso como leña-combustible. Sin embargo, su relativamente mala conformación de fuste, y lento crecimiento respecto a otras especies como *P. pseudostrobus* o *P. patula*, hacen de *P. oocarpa* una especie menos competitiva para aserrío en México (Olvera-Coronel 1985).

En contraste, la especie se destaca por su capacidad para la producción de resina en varios países de América Latina (Perry 1991). En México, justamente su principal uso es la extracción de resina para obtener brea, aguarrás y sus derivados (Gonçalves-de Oliveira 1987). En particular el Estado de Michoacán produce el 94% de las aproximadamente 24,000 toneladas anuales de la producción nacional (SEMARNAT 2017).

A pesar de su gran importancia para la producción de resina y amplia distribución geográfica en México, el interés forestal por esta especie ha sido marginal (Viveros-Viveros et al. 2005). Esto se refleja en la ausencia de plantaciones comerciales y programas de mejoramiento genético para esta especie en México (los ensayos de procedencias en general se encuentran fuera de México; Greaves 1979, Dvorak et al. 2000). Si bien no es una especie amenazada o en peligro de extinción (SEMARNAT 2010), el cambio de uso de suelo está reduciendo de manera importante su distribución natural. En el Estado de Michoacán, por ejemplo, su rango natural de distribución coincide con la de las huertas de aguacate, las cuales se han expandido significativamente a costa de los rodales naturales de *P. oocarpa* (Sáenz-Romero y Tapia-Olivares 2003; Sáenz-Romero et al. 2006; Gutiérrez-Vázquez et al. 2013). Esta paradójica situación subraya la importancia de las poblaciones naturales de *P. oocarpa* como un valioso recurso genético que es necesario conservar (Mendizábal-Hernández et al. 1999, Gutiérrez-Vázquez et al. 2013).

La presión de uso de los rodales naturales de *P. oocarpa* se podría reducir estableciendo plantaciones comerciales en México de uso múltiple: para producción de resina y de madera (Aguiar et al. 2012), tal como se ha desarrollado con éxito en Brasil (Neves et al. 2001). Para hacer las plantaciones comerciales rentables e internacionalmente competitivas, sería necesario incrementar su productividad mediante programas de mejoramiento genético que consideren simultáneamente el mejoramiento de los caracteres relacionados a la producción de resina y de madera. Ello requiere de ensayos de progenies que permitan cuantificar el control genético (heredabilidad) de tales caracteres (Kemp 1973, Svensson et al. 1999, Romanelli y Sebben 2004, Aguiar et al 2012, Gutiérrez et al. 2015).

1.2 Materiales y métodos

Treinta árboles madre fueron seleccionados en rodales naturales con base en el volumen de producción de resina, que estuvieran sanos y que presentaran conos con semilla, tomando como referencia encuestas realizadas a los dueños de las parcelas en aprovechamiento en el Ejido San José de Cañas, Municipio de Ario en el estado de Michoacán, centro-oeste de México. Los árboles candidatos tuvieron un distanciamiento entre ellos de al menos 100 m (Millar y Libby 1991) para disminuir el grado de parentesco (Gutiérrez-Vázquez et al. 2013). La ubicación y características de los árboles seleccionados se detallan en Reyes-Ramos et al. (2019) y en la figura 1.

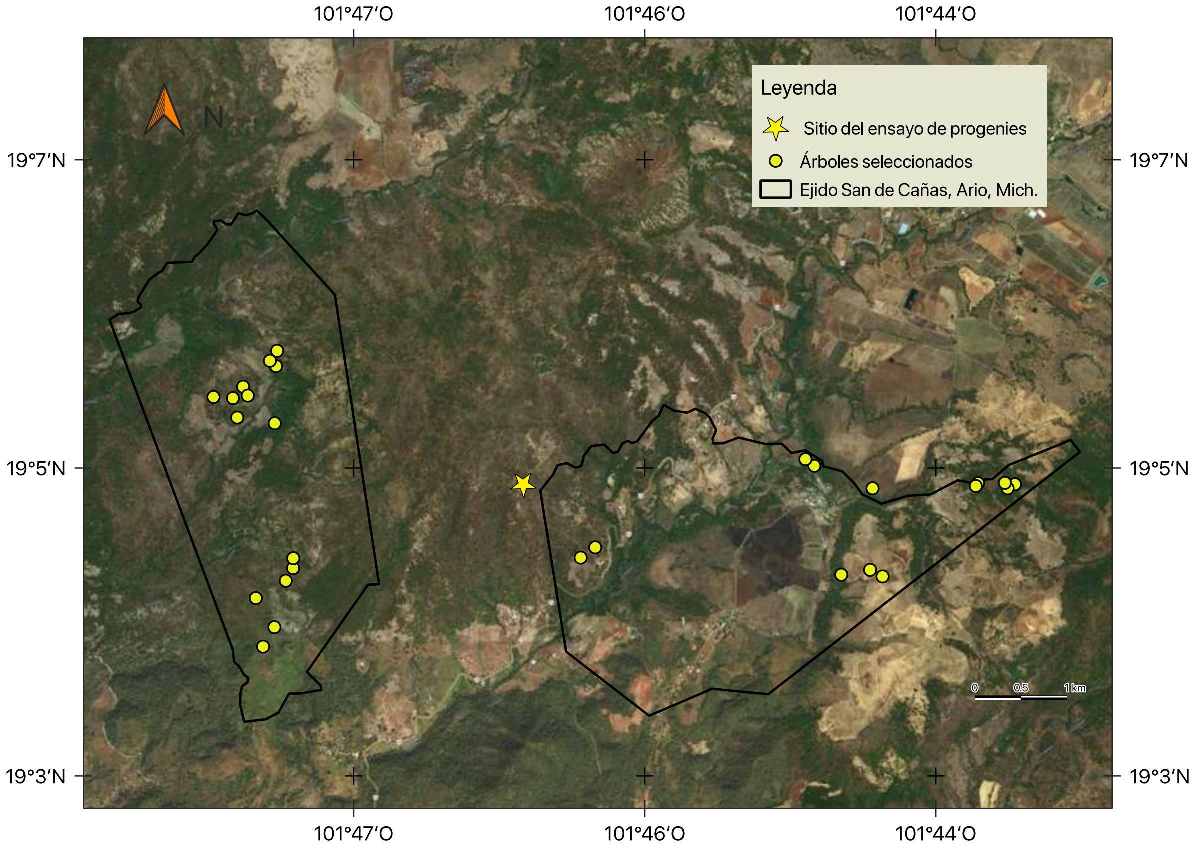

Fig. 1.1 Ubicación de árboles seleccionados y del ensayo de progenies en el Ejido San José de Cañas, Municipio de Ario.

Los conos se recolectaron en el mes de febrero de 2011. Posterior a la extracción de semilla, se aplicó un tratamiento pregerminativo, que consistió en remojo con solución de *Trichoderma* sp. a concentración de 1x10^{11} conidias g^{-1}. Se sembraron las semillas directamente en contenedores de plástico rígido de 170 cm^3 (una semilla por contenedor), manteniendo la identidad de los individuos por familia. Se usó como sustrato una mezcla de turba (50%), vermiculita (20%) y perlita (30%). Las plantas se ordenaron bajo un diseño completamente al azar, en un vivero en Morelia, Michoacán, a una altitud de 1915 m snm, con una temperatura promedio anual de 17.2 ºC, una precipitación media anual de 865 mm, y se mantuvieron en vivero por un periodo de 5 meses. Se le aplicó Trichoderma vía foliar, cada 22 días, con una solución de 20 litros de agua y 60 g de Trichoderma durante los primeros tres meses. Un mes después de la germinación se aplicó fertilizante "iniciador" (N-P-K: 12-61-00) por un periodo de 30 días, posteriormente se aplicó "desarrollo" (N-P-K: 20-07-19) durante tres meses y por último, se aplicó "finalizador" (N-P-K: 4-25-35).

El ensayo de progenies se estableció en campo en julio (inicio de la temporada de lluvias) de 2011, en el Municipio de Ario, Michoacán, a una altitud de 1343 m snm (**Fig. 1.1**). El diseño experimental se organizó en 10 bloques completos al azar, con tres individuos por parcela discontinua. Los árboles se plantaron a marco real a una distancia de 3 x 3 m entre cada planta en un terreno previamente roturado con arado de disco y se fertilizaron al momento de la plantación. En el año uno, tres, cuatro y cinco del ensayo se midieron las variables: altura total (ALT) y diámetro a la base del tallo (DIAM). Mientras que en el año tres, cuatro y cinco se midió el diámetro a la altura del pecho (DAP) a una altura de 1.30 m sobre el nivel del suelo. El DIAM y DAP se midió en los primeros años con un vernier, posteriormente con forcípula y la ALT con una regla y una extensión ajustable. En vivero se obtuvo suficiente planta de 27 familias, con las que se estableció el experimento en campo, sin embargo, durante los primeros años de establecido, dos familias tuvieron baja supervivencia, por lo que se eliminaron de los análisis.

Se obtuvieron las estadísticas descriptivas con el procedimiento MEANS de SAS; el análisis de la varianza se realizó con el procedimiento GLM, obteniéndose los componentes de varianza con el procedimiento VARCOMP a través del método REML (SAS Institute 2016), para cada edad y variable por separado. Antes de realizar los análisis de varianza, los datos se estandarizaron con el procedimiento STANDARD con media igual a cero y varianza igual a uno. El modelo lineal aplicado para el análisis del ensayo fue:

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

donde Y_{ijk} es el valor observado de la *k*-ésima planta de la *j*-ésima familia en el *i*-ésimo bloque; μ es el valor promedio de la población; β_i es el efecto del *i*-ésimo bloque; φ_j es el efecto aleatorio de la *j*-ésima familia [$E(\varphi_j) = 0$; $Var(\varphi_j) = \sigma^2_f$]; $\beta\varphi_{ij}$ es el efecto aleatorio de interacción entre el *i*-ésimo bloque y la *j*-ésima familia [$E(\beta\varphi_{ij})= 0$; $Var(\beta\varphi_{ij}) = \sigma^2_{bf}$]; ε_{ijk} es el error aleatorio experimental [$E(\varepsilon_{ijk}) = 0$; $Var (\varepsilon_{ijk}) = \sigma^2_e$].
El coeficiente de variación genética (CV_g) se calculó mediante la siguiente ecuación (Salaya-Domínguez et al. 2012):

$$CV_g = \left[\frac{\left(\sigma_f^2\right)^{0.5}}{\bar{X}}\right] * 100$$

donde σ^2_f es la varianza de familia y $\bar{x}$ es la media general.

La heredabilidad a nivel de medias de familia se estimó para cada una de las variables mediante las fórmulas propuestas por Zobel y Talbert (1984). Las heredabilidades a nivel individual se estimaron con un coeficiente de determinación genética de tres (Griffin y Cotterill 1988, Hardner y Potts 1995, Farfán-Vázquez et al. 2002, White et al. 2007) para evitar sobreestimar los parámetros genéticos (Namkoong 1966). Se considera que entre las familias de polinización abierta existe una mayor variación debido a que hay una mayor probabilidad de polinización por árboles cercanos (Squillace 1974) y por ello, existe cierta probabilidad de autofecundación (Sorensen y Miles 1974). Por lo anterior, se asumió que la correlación genética entre medios hermanos obtenidos por polinización libre es de 0.33 (Farfán-Vázquez et al. 2002). Las ecuaciones utilizadas en el presente trabajo fueron las siguentes:

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

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

donde $\sigma^2{}_f$ es la varianza de familia; $\sigma^2{}_{bf}$ es la varianza de bloque por familia; $\sigma^2{}_e$ es la varianza del residual; *b* es el número de bloques; *n* es la media armónica del número de individuos en cada unidad experimental. La ecuación utilizada para determinar la media armónica fue:

$$MH = \frac{\boldsymbol{n}}{\Sigma(\boldsymbol{f_i}/\boldsymbol{y_i})}$$

donde *MH* es la media armónica; *n* es el número de datos; f_i es el valor de cada frecuencia; y_i es cada valor observado correspondiente a la variable de interés. En este caso, *b* fue igual a 10 hasta el año cuatro y a nueve en el año cinco, y *MH* varió de 2.29 en el año uno a 1.64 en el año cinco. La heredabilidad de medias de familias se consideró baja si los valores fueron menores a 0.20, media entre 0.20 y 0.50, y alta cuando fue mayor a 0.50 (Stanfield 1971, Pistorale et al. 2008).

El error estándar de la heredabilidad *EE*(h^2) se estimó mediante la fórmula modificada de Jayaraman (1999):

$$EE\ (h_i^2) = (1 - h^2 / 3)\ [1 + nb - 1]\ (h^2 / 3) / [(nb / 2)\ (nb - 1)\ (f - 1)]^{0.5}$$

$$EE\ (h_f^2) = (1\text{-}t)\ (1 + nbt) / [((nb)\ (f - 1))/2]^{0.5}$$

donde *t* es la correlación intraclase (1/3 de la heredabilidad individual), *n* es la media armónica de individuos, *b* es el número de bloques del ensayo, *f* es el número de familias en el ensayo.

Las correlaciones fenotípicas y genéticas entre ALT, DIAM y DAP se calcularon para cada una de las edades y se estimaron con base en el coeficiente de correlación de Pearson. Las correlaciones genéticas se calcularon con la siguiente ecuación (Falconer y Mackay 1996):

$$r_{gxy} = \frac{\sigma_{fxy}}{\sigma_{fx}\,\sigma_{fy}}$$

donde r_{gxy} es la correlación genética entre x e y evaluada sobre los mismos individuos; σ_{fxy} es la covarianza entre x e y, σ_{fx} y σ_{fy} son las desviaciones estándar de familia de las variables x e y. La covarianza σ_{fxy} se estimó con la siguiente ecuación (White y Hodge, 1989):

$$\sigma_{fxy} = \frac{\left[\sigma_{f(x+y)} - \left(\sigma_{fx} + \sigma_{fy}\right)\right]}{2}$$

La ganancia genética se estimó mediante la siguiente fórmula (Diao et al. 2016):

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

donde i es la intensidad de selección, $h^2{}_i$ es la heredabilidad a nivel individual, σ_P^2 es la varianza fenotípica total y $\bar{X}$ es la media de la población.

1.3 Resultados y discusión

La supervivencia promedio del ensayo en el año cinco (2016) fue 59%. El porcentaje de supervivencia de las familias osciló entre 27% y 84%. El número promedio de individuos por familia al final de las evaluaciones fue de 13.8 individuos. Las familias con mayor número de individuos representados en el ensayo fueron dos con 26 y 28 individuos, respectivamente.

Características fenotípicas de las progenies. Las progenies evaluadas en el ensayo alcanzaron en el quinto año, en promedio, una ALT de 5.5 m, un DIAM de 13.4 cm y un DAP de 10.4 cm (**Tabla 1.1**). Los incrementos medios anuales fueron de 1.1 m para ALT, 2.7 cm para DIAM y 2.1 cm para DAP. El coeficiente de variación disminuyó conforme avanzó la edad, de entre el 41.0% y el 40. 7% en la edad uno, en las tres variables, hasta entre el 16.6% y el 20.5% en la edad cinco (**Tabla 1.1**); esto sugiere que con el paso del tiempo el ambiente tuvo cada vez menor influencia sobre el desarrollo del ensayo (Franklin, 1979; Costa y Durel, 1996; Farfán-Vásquez et al. 2002) (**Tabla 1.1**). Las diferencias entre las familias fueron de 0.12 m para ALT el primer año a 1.67 para ALT en el año cinco, de 0.27 cm a 2.85 cm para DIAM y de 1.47 cm a 3.57 cm para DAP (**Tabla 1.1**).

Tabla 1.1 Estadísticas descriptivas para cada variable y año de crecimiento (expresado como subíndice) en campo en un ensayo de progenies de *P. oocarpa* en el Municipio de Ario, Michoacán.

Variable$_{año}$	***$\bar{X}$±ErrEst***	***Mínimo***	***Máximo***	***DesEst***	***CV***	***AmpFam***
ALT_1	0.22±0.00	0.04	0.56	0.09	40.97	0.12
ALT_3	2.22±0.03	0.70	4.30	0.57	25.46	0.63
ALT_4	3.53±0.03	1.40	5.60	0.68	19.19	0.95
ALT_5	5.51±0.05	2.30	7.95	1.00	18.07	1.67
DIAM_1	0.54±0.01	0.19	1.51	0.22	40.67	0.27
DIAM_3	6.37±0.07	0.94	9.52	1.46	22.99	1.92
DIAM_4	9.93±0.08	3.41	14.92	1.72	17.35	2.32
DIAM_5	13.44±0.12	5.00	19.20	2.23	16.59	2.85
DAP_3	2.69±0.05	0.63	6.31	1.08	40.03	1.47
DAP_4	6.15±0.08	1.42	10.36	1.68	27.25	2.41
DAP_5	10.38±0.11	4.00	16.40	2.13	20.48	3.57

$\bar{X}$±ErrEst = valor promedio±error estándar; DesEst = desviación estándar; CV = coeficiente de variación

±ErrEst = valor promedio±error estándar; DesEst = desviación estándar; CV = coeficiente de variación; AmpFam = amplitud de familias (el mayor valor promedio de familia menos el menor valor promedio de familia) ALT_1-ALT_5, DIAM_1-DIAM_5, DAP_3-DAP_5 =altura total (m), diámetro a la base del tallo (cm) y diámetro normal (cm, a la altura de 1.30 m del nivel del suelo) en los diferentes años después de la plantación en campo (1, 3, 4, 5).

Las dos mejores familias a partir del tercer año para ALT fueron las familias 45 y 61; en DIAM y DAP se observó lo mismo que en ALT; a partir de la edad de tres años, la mejor familia para DIAM y DAP fue la familia 45 (**Tabla 1.2**).

Tabla 1.2 Valores de las características promedio de crecimiento de las tres mejores familias de un ensayo de progenies de *P. oocarpa* en el Municipio de Ario, Michoacán.

Edad	**ALT (m)**			**DIAM (cm)**			**DAP (cm)**		
(años)	**Fam**	**Prom±err**	**CV%**	**Fam**	**Prom±err**	**CV%**	**Fam**	**Prom±err**	**CV%**
1	65	0.31±0.04	38.57	60	0.68±0.19	61.74	-	-	-
1	14	0.30±0.08	43.23	65	0.63±0.05	21.52	-	-	-
1	15	0.26±0.02	33.95	09	0.62±0.06	48.90	-	-	-
3	61	2.60±0.16	27.40	45	7.42±0.17	3.17	45	3.56±1.28	50.85
3	09	2.58±0.16	28.03	14	7.23±0.92	12.74	09	3.37±0.27	36.18
3	45	2.57±0.77	42.37	61	6.90±0.33	21.38	40	3.31±0.59	50.67
4	45	4.05±0.95	33.17	45	11.26±1.36	17.13	45	7.73±1.77	32.38
4	09	4.00±0.19	21.91	55	10.69±0.25	11.05	09	6.93±0.40	26.51
4	61	3.91±0.15	17.15	12	10.68±0.38	17.08	14	6.83±0.65	16.38
5	45	6.58±1.08	23.12	45	14.60±1.40	13.56	45	12.45±1.55	17.61
5	61	6.06±1.93	14.23	19	14.53±0.42	11.27	19	11.43±0.40	13.43
5	19	5.95±1.50	9.75	67	14.29±0.32	10.47	64	11.26±0.50	14.04

La comparación del crecimiento promedio entre familias con otros ensayos previos resulta difícil, en el sentido de que no siempre se reportan edades comparables, y la influencia del clima y suelo del sitio de ensayo son determinantes. Dicho lo anterior, se podría decir que los promedios entre familias del presente ensayo a los cinco años de edad a la plantación, fueron similares en ALT a los obtenidos en el año seis en procedencias de *P. oocarpa* originarias de Guatemala, Honduras, Nicaragua y Belice evaluadas en Tanzania (5.7 m, Mugasha et al. 1996), aunque inferiores en ALT y DAP (en un 35% y 30%, respectivamente) en comparación con un ensayo de procedencias de *P. oocarpa* de Honduras y Guatemala a la misma edad (cinco años), ensayadas en Brasil (Moura et al. 1998).

Componentes de la varianza. Se detectaron diferencias significativas (*P≤0.05*) entre las familias, en todas las variables estudiadas, a partir de los tres años, con excepción de la variable DIAM a los tres años de edad. Esto indica que existe variabilidad genética significativa entre las familias evaluadas (**Tabla 1.3**), y por tanto, que es factible obtener ganancias genéticas a partir de la selección de los mejores genotipos no antes de los tres años de edad a la plantación (Resende et al. 1991).

La varianza de familia ($\sigma^2_{f)}$ para DIAM y DAP, incrementa su contribución a la varianza total conforme se incrementa la edad, hasta llegar a 6.8 % y 5.2 %, respectivamente (**Tabla 1.3**). Si el diámetro del árbol es un carácter asociado positivamente al potencial de producción de resina (Squillace 1965), estos resultados sugieren que es recomendable realizar selección de los individuos o familias con base en el diámetro (basal o a la altura del pecho) a la edad de cinco años.

Como es común en ensayos de progenie de medios hermanos, en los que existe una gran variación entre individuos de la misma familia, la varianza del error (σ^2_e) fue el componente que tuvo mayor contribución a la varianza total para las tres variables en todas las edades (**Tabla 1.3**). Esto eventualmente permitiría hacer una selección de mejores individuos dentro de familias (Farfán-Vázquez et al. 2002).

Tabla 1.3 Coeficiente de variación genética y componentes de varianza de un ensayo de progenies de *P. oocarpa* en el Municipio de Ario, Michoacán.

Variable$_{año}$	**Pr > F**	n_{fam}	n_{bloq}	CV_g %	CV_p %	σ^2_f %	σ^2_e %
ALT$_1$ (m)	0.46	2.29	10	0	41	0.1	92.5
DIAM$_1$ (cm)	0.57	2.29	10	0	40.6	0	90.2
ALT$_3$ (m)	0	1.76	10	5.9	25.6	6.3	74.5
DIAM$_3$ (cm)	0.2	1.74	10	2.7	23.6	1.5	81.8
DAP$_3$ (cm)	0	1.64	10	6.3	39.7	2.4	78.6
ALT$_4$ (m)	0	1.74	10	5.1	19.3	7	76.8
DIAM$_4$ (cm)	0.04	1.74	10	3.8	17.7	4	86.7
DAP$_4$ (cm)	0	1.75	10	5.6	28	4.6	82.5
ALT$_5$ (m)	0.05	1.69	9	3.6	18.2	4.2	75.1
DIAM$_5$ (cm)	0	1.68	9	3.9	16.7	6.8	80.1
DAP$_5$ (cm)	0.02	1.68	9	4.4	20.5	5.2	81.7

ALT = altura total; DIAM = diámetro basal; DAP = diámetro a la altura del pecho (1.30 m); Pr > F = significancia de la prueba de hipótesis para la diferencia entre familias; n_{fam} = media armónica del número de individuos por familia por bloque; n_{bloq} = número de bloques; CV_g = coeficiente de variación genética en porcentaje; *CV %*= coeficiente de variación en porcentaje; σ^2_f % = varianza de familias en porcentaje de contribución a la varianza total; σ^2_e % = varianza del error en porcentaje.

Heredabilidad individual y de media de familias. Las heredabilidades, tanto a nivel individual (h^2_i) como de media de familias (h^2_f), en general aumentaron con la edad (**Tabla 1.4**). Por ejemplo, DIAM a la edad de cinco años tuvo valores muy promisorios para realizar selección con base en ese carácter y edad: $h^2_i = 0.22$ y $h^2_f = 0.54$. Por ello, se confirma la tendencia de los datos que sugieren que es recomendable realizar la selección a la edad de cinco años con base en el diámetro.

Tabla 1.4 Heredabilidades en diferentes edades (1, 3, 4 y 5 años) en un ensayo de progenies de *P. oocarpa* en el Municipio de Ario, Michoacán.

Variable$_{año}$	**h^2_i**	**EE h^2_i**	**h^2_f**	**EE h^2_f**
ALT_1	0.003	0.013	0.03	0.062
$DIAM_1$	0.00	0.013	0.00	0.060
ALT_3	0.21	0.034	0.54	0.124
$DIAM_3$	0.05	0.022	0.23	0.088
DAP_3	0.08	0.025	0.29	0.099
ALT_4	0.23	0.036	0.58	0.150
$DIAM_4$	0.13	0.028	0.42	0.115
DAP_4	0.13	0.028	0.47	0.117
ALT_5	0.15	0.032	0.44	0.124
$DIAM_5$	0.22	0.038	0.54	0.146
DAP_5	0.17	0.034	0.47	0.130

Edad = fecha de medición de variables de crecimiento, h^2_i = heredabilidad individual; EE (h^2_i) = error estándar; h^2_f = heredabilidad de medias de familia.

En concordancia con la falta de significancia de la variación entre familias a la edad de un año (**Tabla 1.3**), la heredabilidad a esa edad tuvo un valor de cero en DIAM y en ALT presentó valores muy bajos ($h^2_i = 0.003$; $h^2_f = 0.03$). Esto puede deberse a que a temprana edad aún no se expresa plenamente el potencial de crecimiento de cada familia y al proceso de adaptación después de la plantación en campo (Cotterill y Dean 1988). El mismo patrón de variación ha sido observado en *P. ponderosa* (Namkoong y Conkle 1976), Pseudostuga menziesii (Franklin 1979) y *P. radiata* (Cotteril y Dean 1988).

Los resultados de h^2_i obtenidos en este estudio fueron similares a los obtenidos en ensayos de progenies de otras especies forestales, para los que en general se han reportado valores de h^2_i entre 0.10 y 0.30 (Cornelius 1994, White et al. 2007). Por ejemplo, los valores encontrados para ALT a los cinco años de edad ($h^2_i = 0.15$ y $h^2_f = 0.44$), son similares a los reportados para *P. radiata* ensayado en Orozko, España, a los seis años de edad ($h^2_{i\,\mathrm{ALT}} = 0.15$) (Arregui et al. 1999); *P. caribaea* Morelet de cinco años de edad establecido en Chaguamaras, Venezuela (Vásquez y Dvorak 1996) y otro de cinco años establecido en El Amparo, Colombia ($h^2_{i\,\mathrm{ALT5}} = 0.24$); *P. chiapensis* (Mart.) Andresen en Los Guadales, Colombia ($h^2_{i\,\mathrm{ALT5}} = 0.28$); *P. tecunumanii* (Schw.) Eguiluz et Perry en San José, Colombia y Wilgeboom, Sudáfrica ($h^2_{i\,\mathrm{ALT5}} = 0.32$; $h^2_{i\,\mathrm{ALT5}} = 0.41$) (Vásquez y Dvorak, 1996); *P. patula* Sitio 2 en Acoxochitlán, Puebla ($h^2_{i\,\mathrm{ALT5}} = 0.33$) (Salaya-Domínguez et al. 2012) y en Sitio 1 Aquixtla, Puebla ($h^2_{i\,\mathrm{ALT5}} = 0.09$) (Salaya-Domínguez et al. 2012).

Los valores de h^2_f presentaron un patrón similar al observado en la h^2_i (**Tabla 1.4**), con valores más altos que ésta última, y con una tendencia a incrementarse con la edad. Algunos estudios han demostrado que la h^2_f se incrementa con la edad, debido a la acumulación del crecimiento derivado de la expresión de un potencial de crecimiento diferencial ente familias, medible hasta antes de que la competencia impida una cuantificación robusta de tal diferenciación (Namkoong y Conkle 1976; Franklin 1979; Costa y Durel 1996; Ignacio-Sánchez et al. 2005). Los valores de h^2_f de *P. oocarpa* en este estudio fueron mayores comparados con los valores estimados para *P. patula* en dos sitios ($h^2_{f\,\mathrm{ALT5}} = 0.17, 0.27$; $h^2_{f\,\mathrm{DAP}} = 0.19, 0.25$) (Salaya-Domínguez et al. 2012). Los valores de h^2_f sugieren que hay un importante potencial de selección entre las familias (Sebben et al. 2008). En promedio los valores de heredabilidad más altos se observaron para las características del diámetro, lo cual indica que este será un rasgo importante en la selección temprana.

Correlaciones fenotípicas y genéticas entre caracteres. Las correlaciones fenotípicas entre cada par de variables en el caso de *P. oocarpa* fueron de medias a altas y en todos los casos positivas. Los valores de correlación más altos se observaron entre DIAM con DAP a la edad de cinco años ($r_{f\,5\,(DIAM\text{-}DAP)}$ = 0.86) y entre ALT con DAP a la edad de tres y cuatro años ($r_{f\,3\,(ALT\text{-}DAP)}$ = 0.82, $r_{f\,4\,(ALT\text{-}DAP)}$ = 0.81), lo que demuestra una cercanía entre los pares de variables (**Tabla 1.5**).

Tabla 1.5 Correlaciones fenotípicas (r_{fxy}) y genotípicas (r_{gxy}) entre variables de crecimiento.

Correlaciones	Edad	ALT-DIAM	DIAM-DAP	ALT-DAP
r_{fxy}	1	0.31	.	.
	3	0.60	0.53	0.82
	4	0.68	0.75	0.81
	5	0.68	0.86	0.77
r_{gxy}	1	0	.	.
	3	0.91	0.72	0.95
	4	0.72	0.86	0.92
	5	0.74	0.96	0.95

r_{fxy} = correlación fenotípica; r_{gxy} = correlación fenotípica; ALT = altura total; DIAM = diámetro basal; DAP = diámetro normal (1.30 m).

Las correlaciones genéticas en *P. oocarpa* en este estudio fueron altas y positivas, lo cual confirma que existe una fuerte asociación entre los caracteres de crecimiento evaluados y que la selección para una de las características tendrá un impacto positivo en mejorar otra (Resende et al. 1991, White et al. 2007, Macedo et al. 2013). El grado de correlación fenotípica (r_f = 0.86) y genotípica (r_g = 0.96) más alto a la misma edad, se encontró para la asociación DIAM y DAP en el quinto año del ensayo; esto probablemente debido al alto grado de relación que naturalmente presentan entre sí ambos caracteres, derivado del factor de forma del fuste. Sin embargo, en promedio el grado de correlación genética fue más alto para la asociación entre ALT y DAP ($r_{g\ 3,\ 4,\ 5\ (\text{ALT-DAP})}$ = 0.92 a 0.95; **Tabla 1.5**). Esta correlación sugiere que entre las dos características la interacción genotipo por ambiente fue menor (Paludzyszyn-Filho et al. 2002). Los valores observados coinciden con la mayoría de los estudios de especies forestales que han demostrado una fuerte correlación genética entre estas este par de variables (Arregui et al. 1999, Farfán-Vázquez et al. 2002, Morales-González et al. 2013).

En general, las correlaciones genéticas fueron superiores a las respectivas correlaciones fenotípicas, lo que indica que la contribución de los factores genéticos en la expresión de los caracteres fue mayor en comparación con los factores ambientales (Fernando-Amabile et al. 2015). Por otro lado, dado que la alta capacidad de producción de resina y las características de crecimiento también parecen ser variables genéticamente correlacionadas (Rodrigues et al. 2008, Zheng et al. 2013, Lai et al. 2017) se puede suponer que no solamente se logrará una mejora en la producción de resina, sino que se puede realizar una selección para lograr ganancias en la productividad de madera. La selección de DAP en este caso es más deseable que la selección con base en DIAM o ALT, ya que es el carácter de crecimiento que está más probablemente relacionado con el volumen producción de resina (Squillace 1965, Rodrigues et al. 2008, Zheng et al. 2013). Esto coincide con lo encontrado por Squillace (1965) en árboles de 19 años de edad de *P. elliottii* que la correlación genética entre ambas variables permitía aumentar el rendimiento de resina al doble y al mismo tiempo lograr una ganancia de 6% en el crecimiento del DAP.

Respuesta a la selección*.* En el presente estudio se determinó seleccionar 20% de los árboles con base en las características de crecimiento evaluadas con un índice de selección de *i* = 1.4. Los resultados sugieren que la edad óptima para la selección de la característica de DAP es el año cinco y que tomando en cuenta la alta heredabilidad obtenida y la variación fenotípica, se podría esperar un aumento de 4.95% en esta característica en la misma edad en la próxima generación (**Tabla 1.6** Ganancia genética (%) esperada, con una intensidad de selección de i = 1.4, para variables de crecimiento en un ensayo de progenies de medios hermanos de P. oocarpa en el Municipio de Ario, Michoacán.).

Tabla 1.6 Ganancia genética (%) esperada, con una intensidad de selección de i = 1.4, para variables de crecimiento en un ensayo de progenies de medios hermanos de P. oocarpa en el Municipio de Ario, Michoacán.

Edad	ALT	DIAM	DAP
1	0.12	0.00	.
3	7.61	1.63	4.48
4	6.26	3.17	5.88
5	3.85	5.19	4.95

ALT = altura total; DIAM = diámetro basal; DAP = diámetro a la altura del pecho (1.30 m).

Para el DIAM se esperaría un aumento similar de 5.19%. En el caso de la ALT los resultados indican que se obtendría un 4% de ganancia en la siguiente generación. En coníferas estimar los valores de la respuesta a la selección a la edad de cinco años puede parecer una edad relativamente joven, dada la longevidad de las especies (White et al. 1993, Wu 1999). Sin embargo, diversos estudios con otras especies demuestran la existencia de correlación alta entre las ganancias genéticas estimadas a edades tempranas y ganancias obtenidas al final del turno (White et al. 1993, Wu 1999).

1.4 Conclusiones

El presente ensayo de progenies permitió detectar una variabilidad genética significativa entre familias de medios hermanos en los caracteres de altura (ALT), diámetro a la base del fuste (DIAM) y diámetro a la altura del pecho (DAP). La existencia de una variación genética aditiva significativa entre familias, indica que es viable obtener ganancias genéticas para esos caracteres mediante selección de mejores individuos y/o de mejores familias. La edad óptima de selección podría ser el cuarto año de edad (posterior a la plantación en campo) para ALT y el quinto año para DAP y DIAM. Considerando que trabajos previos han demostrado una asociación positiva entre DAP y producción de resina, DAP sería la característica en la que se enfocaría la selección para incrementar la producción de madera: Al tener ganancias genéticas en DAP (del orden del 4 %), sería de esperar tener un incremento en la producción de resina. Una limitación de este estudio es que el ensayo se realizó en un solo sitio; futuros ensayos requerirán de varios sitios de campo, para cuantificar la magnitud del efecto del medio ambiente y de la interacción genotipo por medio ambiente, idealmente con un mayor número de familias ensayadas

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Capítulo 2 : Description of the defensive anatomical traits and its genetic variation in wood and bark of *Pinus oocarpa*

Descripción de las características anatómicas de defensa y su variación genética en la madera y corteza de *Pinus oocarpa*

Resumen

A pesar de su amplia distribución y los diferentes usos de *P. oocarpa* en México, el conocimiento sobre la variación genética y los estudios sobre los parámetros genéticos de las estructuras defensivas de la especie son poco frecuentes. El presente estudio intenta proporcionar una evaluación exhaustiva de la morfología de los canales resiníferos de árboles madre y sus progenies en un ensayo de polinización abierta de *P. oocarpa*. Los canales resiníferos son estructuras fundamentales de los mecanismos de defensa de las coníferas, los cuales varían según la edad del árbol, el genotipo, la especie y también pueden estar influenciados por factores ambientales. En el presente estudio se exploró la variación intraespecífica del sistema de canales resiníferos en los tejidos vasculares secundarios (madera y corteza interna) de árboles de *Pinus oocarpa*. Se estudió la varianza entre familias y el posible control genético de estas estructuras defensivas, bajo la hipótesis de que las características de los canales resiníferos presentarían variaciones influenciadas por la genética del árbol. Analizamos muestras de dos grupos de árboles, el primer grupo se formó con las muestras de los árboles madre seleccionados como superiores en producción de resina y el segundo grupo, con muestras de sus progenies (árboles de seis años de edad) establecidas como parte de un ensayo. Los canales axiales y radiales mostraron diferencias en su tamaño y número, entre tejidos (madera y corteza) y entre edades (adultos y juveniles). Los resultados obtenidos sugieren que existe una baja variación intraespecífica para todas las características analizadas, con la excepción de la densidad los canales axiales. La densidad de los canales axiales fue la única característica que mostró diferencias entre genotipos para realizar estudios de heredabilidad y otros parámetros genéticos. Por lo tanto, este rasgo es probablemente el único susceptible de ser explorado como parte de un programa de mejoramiento genético.

Palabras clave: anatomía, canales resiníferos axiales, canales resiníferos radiales, floema secundario, variación fenotípica, xilema secundario

Abstract

Despite the extensive distribution and use of *P. oocarpa* in Mexico, knowledge on the range of genetic variation and magnitude of quantitative parameters of the defensive structures of the species is rare. Our study attempts to fill this gap by providing a comprehensive assessment of resin duct traits in mother trees and their offspring's in an open-pollinated trial of *P. oocarpa*. Resin ducts are fundamental structures of the defense mechanisms of the coniferous trees. They vary depending on the age of the tree, the genotype, the species, and may be influenced by environmental factors. We investigated intraspecific variation of the resin duct system in secondary vascular tissues (wood and inner bark) of *P. oocarpa* trees. Our study attempts to explore the variance among families and the possible genetic control of these defensive structures. We hypothesized that those resin ducts features would vary influenced by the tree´s genetic. We analyzed samples from two groups of trees. First, from mature mother trees selected as superior in resin yield. Second, from their six-year-old offspring´s established in a progeny trial. Axial and radial ducts showed differences in their size and quantity, between wood and bark and between ages. The results presented low intraspecific variation for all the analyzed features except for the axial duct density. Therefore, this trait is likely to be explored as part of a tree breeding program. The axial duct density was the only characteristic that showed differences among genotypes to carry out heritability studies and other genetic parameters.

Keywords: anatomy, axial resin ducts, radial resin ducts, secondary phloem, phenotypic variation, secondary xylem

2.1 Introduction

The ecological success of conifers for millions of years is mainly due to the capacity these trees have developed to defend themselves against the bark beetles, fungi, and its associated phytopathogens (Krokene 2015) along with their common long lifespan. Its primary defense mechanism is the synthesis of oleoresin (Krokene and Nagy 2012) or pine resin (hereinafter, resin). Resin is a viscous and sticky substance of an outstanding chemical diversity (Angyalossy et al. 2016), soluble in organic solvents. It is a physical/mechanical as well as a chemical defense mechanism (Ferrenberg et al. 2015). Its primary function is to trap the attacking invertebrate herbivores and seal wounds (Krokene and Nagy 2012).

Resin ducts are multicellular structures paved by layers of epithelial cells that function in secretion and in storage of resin (Angyalossy et al. 2016). The epithelial cells are the site of resin biosynthesis (Phillips 2012) and secrete the resin into the lumen of the duct in a granulocrine way (Beck 2010). Duct formation can be a common feature of the plant development in the primary tissues such as the cortex and in secondary xylem, and phloem (inner bark) of the aerial and subterranean organs (Beck 2010). Resin ducts differentiate as constitutive defense, and so-called traumatic ducts can be induced by external abiotic factors (da Silva Rodrigues-Corrêa et al. 2013). There are two types of resin ducts in the secondary xylem (wood), the axial resin ducts, oriented parallel to the tracheids and the radial resin ducts, which extend radially through the rays (Beck 2010). Genetic and environmental factors control their formation and structure (Larson, 1994; Boschiero and Tomazzello-Filho 2012; Westbrook et al. 2015).

The anatomy of the resin duct system also varies according to the species (Boucher et al. 2001). It also has diagnostic value for the identification of coniferous wood (Panshin and de Zeeuw 1980). Resin duct traits are also important in relation to productivity of resin (Schopmeyer et al. 1954; Rodríguez-García et al. 2014; Neis et al. 2019b). They may play an essential role as predictors of tree survival against bark beetles (Kane and Kolb 2010; Ferrenberg et al. 2014; Hood et al. 2015; Zhao and Erbilgin 2019) and could be a valuable management tool for forest tree programs (Rodríguez-García et al. 2014).

Nowadays, it is well-known pine resin productivity can be increased through breeding (Bhat et al. 2016). However, research on the anatomy of resin ducts is still needed, as it can be one of the strategic traits that could help to understand the potential productivity of the pine resinous species (Boschiero and Tomazzello-Filho 2012; Rodríguez-García et al. 2014; Neis et al. 2019b). Currently, pine resin terpenes are used as key raw materials in a variety of products, such as adhesives, inks, fragrances, agrochemicals, flavor enhancers, and pharmaceutical products (Neis et al. 2019a) and are gaining more importance as renewable, green chemicals that could replace petroleum-based chemicals and fuels (da Silva Rodrigues-Corrêa et al. 2012).

There are several studies that describe the resin ducts in the wood of several species of European, Asian and North American pine trees (Werker and Fahn 1969; Hodges et al. 1981; DeAngelis et al. 1986; Zhenghai and Ruching 1987; Blanche et al. 1992; Wu and Hu 1997; Wiedenhoeft et al. 2003; Esteban et al. 2005; Zhang et al. 2008; Ananías et al. 2010, Neis et al. 2019b; Zhao and Erbilgin, 2019; Govina et al. 2020). Most of the studies cover mainly four species: *P. taeda*, *P. pinaster*, *P. sylvestris*, and *P. radiata* (**Supplementary Table 2.1**). In some coniferous species like *Picea abies*, resin duct traits were found to be under strong genetic control (Rosner and Hannrup 2004). However, in *P. elliottii*, *P. pinaster*, and *P. radiata*, it has been observed that the number of resin ducts per surface area was the only trait that had enough variation for breeding (Westbrook et al. 2015; Peter 2018, Govina et al. 2020).

Pinus oocarpa Schiede ex Schltdl. is a pine species from the subdivision Oocarpae (Gutiérrez-Vázquez et al. 2013). It has a wide distribution from northern Mexico to Nicaragua (Farjon and Filer 2013). The species is used for timber plantations (Gutiérrez-Vázquez et al. 2013) and resin tapping (CONAFOR 2013). According to Gutiérrez-Jarquín (1977), the state of Michoacán is the leading resin producer in Mexico (SEMARNAT 2019) and *P. oocarpa* is the second-highest yielding species.

In Mexico, pine resin is used since ancient times (Martínez-Cortés 1970) and resin-tapping activities have always played an important role in the welfare of forest communities (CONAFOR 2013). Forest communities collectively hold an estimate of 60% of the national forest estate (Hodgdon et al. 2013) some of whom live marginally at the edge of subsistence (CONAFOR 2013). However, the intensive extraction, as we know it today, began since the second decade of the 20th century (Romahn-de la Vega 1982). Currently, it generates an annual income of 21,273,000 USD (SEMARNAT 2017).

Even though there is a long history of resin exploitation in Mexico, there are only a few studies about the anatomy of resin ducts and no data on their heritability, since research mainly focuses on its industrial transformation or the extraction methods (CONAFOR 2013). Descriptive wood anatomical studies of 25 *Pinus* species record some data on resin ducts (Huerta 1963; Olvera-Coronel 1981; Olvera-Coronel 1985; Pérez-Olvera and Dávalos-Sotelo 2016). At present, knowledge of how the genetic and environmental effects influence the anatomy of resin ducts of the pine species in Mexico is scarce. So, our questions were how the resin ducts vary among tissues, ages, and families and what is the magnitude of the genetic parameters.

Therefore, this study aimed to a) describe and quantify the intraspecific variation of resin duct traits in the wood and inner bark of *P. oocarpa* b) explore the differences between juvenile and mature trees, and c) estimate the genetic parameters and the response to selection. All this under the hypothesis, that the size of the resin ducts is a less variable trait, and density has a greater phenotypical variation, and thus a stronger genetic control.

2.2 Materials and methods

Collection of samples. We collected wood cores from two different groups of trees. The first group comprised a selection of 24 mother trees or families of *P. oocarpa* selected in the Ejido San José de Cañas, municipality of Ario, state Michoacán in Mexico. The trees were phenotypically superior in resin yield, with an average age of 66 years (±20), and a mean breast height diameter of 62.14 cm (±11.55). All mother trees were under tapping with two tapping cuts on average.

The second group of cores was taken from 74 individuals from a six-years-old progeny trial from eight families. The trial was established with seedlings grown from open-pollinated seeds collected from the mother trees mentioned above in the same municipality in a nearby locality in 2011. Because these progenies have grown in a single site, the intraspecific variability could be studied with a minimal influence of environmental factors (Martín et al. 2010). From the mother trees, we took one sample per tree on the upper part of the face that was being tapped, with a punch of 1.27 cm in diameter at breast height (approx. 1.30 m). From the trees of the progeny test, we took the cores 30 cm from the ground.

Microscopic and macroscopic analyses. Each sample (bark, vascular cambium, and wood) was fixed in formalin-glacial acetic acid-ethyl alcohol (Ruzin 1999) right after it was taken in the field. Then, we stored the samples in a solution of glycerol, water, and ethyl alcohol (1: 1: 1) until we started the microtechnique. Transverse and serial tangential sections 20-30 µm thick were made with a sliding microtome (Leica SM2000R, Germany). The sections were stained with safranin and Fast-green and mounted with synthetic resin (Hycel de México, Mexico). Transverse and tangential sections were photographed at different magnifications (4X to 20X) with a digital camera (Evolution LC Color, USA) adapted to an Olympus BX51 microscope (Olympus, Japan). Later we analyzed the anatomical characteristics with the software "Fiji," ImageJ Version: 2: 0.0-rc-69/1.52i. Additionally, we also photographed the transverse planes of each sample with a Axio Zoom V16 microscope (Zeiss, Germany) to have a general view of the distribution of axial resin ducts in various growth rings.

Characteristics evaluated. We studied two types of resin ducts: (a) the axial ducts in the transverse plane of the secondary xylem (hereafter AD) and (b) radial ducts in the tangential plane of both the secondary xylem and phloem (hereafter RDX, and RDP, respectively). We quantified the lumen area (size) of the ducts (only epithelium and lumen - method A), the Feret´s diameter of the lumen, and the number of ducts per mm^2 (density) for all the ducts. Also, we measured the standard diameter of the axial ducts (method A), the diameter of the complex of the axial ducts (method B) (Richter et al. 2004), and counted the number of epithelial cells per duct, the number of subsidiary cells per axial duct, and the area of the tangential face of the calcium oxalate crystals in the phloem (**Table 2.1**). We differentiated between constitutive and induced resin duct formations and did not include tangential bands of axial resin ducts (i.e. three or more ducts in a row), which we considered as traumatic resin duct formation.

Table 2.1 Description of resin duct parameters measured in *P. oocarpa* progenies and mother trees.

Trait	***Unit***	***Description***
AD area	mm^2 (method A)	lumen area of the ducts in xylem tissue (epithelium and lumen)
RD area	mm^2	lumen area of the ducts in each tissue (epithelium and lumen)
Feret´s diameter	mm	mean diameter of all ducts (epithelium and lumen) in each tissue
Normal diameter	mm	mean diameter of the axial ducts in xylem (epithelium and lumen)
Diameter duct complex	mm (method B)	mean diameter (epithelium, lumen, and subsidiary cells)
AD density	no./mm^2	number of ducts per mm^2 in the xylem
RD density	no./mm^2	number of ducts per mm^2 in each tissue
RD diameter	mm	mean diameter (epithelium and lumen)
ECA per axial duct	cells/axial duct	number of epithelial cells per axial duct
ECR per radial duct	cells/radial duct	number of epithelial cells per radial duct in each tissue
Subsidiary cells per duct	cells/axial duct	number of subsidiary cells per axial duct in xylem tissue
Calcium oxalate crystals	mm^2	area of crystals (area of the tangential face)

Statistical analyses. The descriptive statistics of each one of the studied anatomical characteristics were obtained. However, due to the fragility of the tissue, the number of individuals per family considered in the statistical analyses was variable (progenies median = 10, mother trees = 1). All the analyses were performed with SAS University Edition software version 9.4. To analyze the differences of the resin duct features between tissues we performed a T-test. The possible differences between trees/families were estimated through an analysis of variance (ANOVA) following this model:

$$Y_{ij} = \mu + F_i + \varepsilon_{ij}$$

where Y_{ij} is the observed value of the j^{th} plant of the i^{th} family, μ is the general mean; F_i is the effect of the i^{th} family, and ε_{ij} is the experimental random error. Variance components for the progenies were estimated using the PROC VARCOMP, via the REML method. A one-way analysis of variance was used to test for variation in number and size of resin ducts among families. Duncan's Multiple Range Test (Steel and Torrie 1980) was used to test for differences among families.

The difference of the anatomical characteristics between ages (mothers and progenies) was estimated by a two-way analysis of variance with the PROC GLM procedure following this model:

$$Y_{ijk} = \mu + F_i + A_j + \varepsilon_{ijk}$$

where Y_{ijk} is the observed value of the k^{th} plant of the j^{th} family in the i^{th} age, μ is the average value of the population; F_i is the effect of the i^{th} family, A_j is the random effect of the j^{th} age [E (φ_j) = 0; Var (φ_j) = $\sigma^2{}_f$]; ε_{ijk} is the experimental random error [E (ε_{ijk}) = 0; Var (ε_{ijk}) = $\sigma^2{}_e$].

The narrow-sense heritability ($h^2{}_i$) and the heritability at family mean level ($h^2{}_f$) were estimated using the following formulae adapted from Zobel and Talbert (1992) using a lower coefficient of determination in order not to overestimate the results (Namkoong, 1966):

$$h^2{}_i = 3\, \sigma^2{}_f / (\sigma^2{}_f + \sigma^2{}_e)$$

$$h^2{}_f = \sigma^2{}_f / [\sigma^2{}_f + (\sigma^2{}_e / n)]$$

where $\sigma^2{}_f$ is the family variance; $\sigma^2{}_e$ is the residual variance, n number of individuals within the family. The standard error of the heritability estimates SE (h^2) was calculated with the following formulae adapted from Jayaraman (1999):

$$SE\ (h_i^2) = (1 - h^2 / 3)\ [1 + n - 1]\ (h^2 / 3) / [(n / 2)\ (n - 1)\ (f - 1)]^{0.5}$$

$$SE\ (h_f^2) = (1-t)\ (1 + nt) / [((n)\ (f - 1))/2]^{0.5}$$

where *t* is the intraclass correlation (1/3 of the narrow-sense heritability), *n* harmonic mean of the individuals. The genetic gain was estimated by the following formula (Diao et al. 2016):

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

where *i* symbolizes selection intensity (1.4), h^2_i narrow-sense heritability, σ_P^2 total phenotypical variance y $\bar{X}$ the mean of the population.

2.3 Results

Genetic parameters and heritability. Inspection of a box plot allowed recognizing outliers; normality was assessed by the Shapiro-Wilk normality test for each design cell. There were no significant outliers and the residues were normally distributed ($p > 0.05$). In the progenies, statistically significant differences were found among families only for the number of AD (density) (**Fig. 2.1, Table 2.2**).

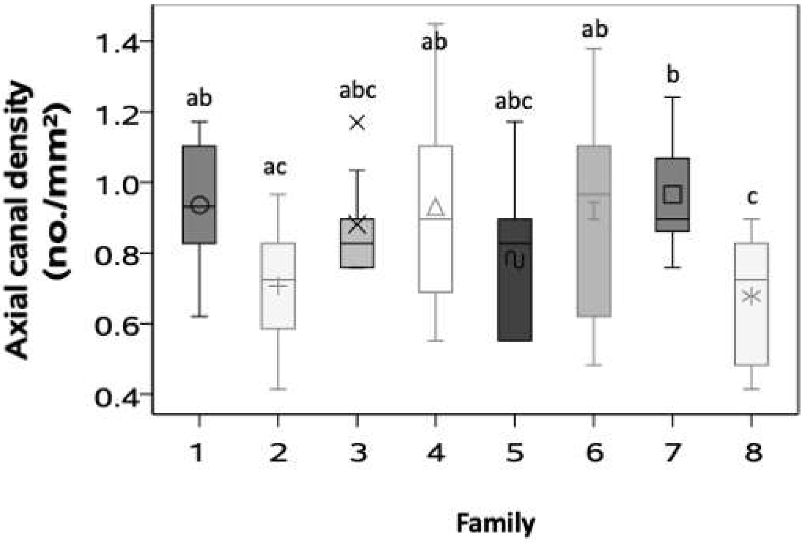

Fig. 2.1 Phenotypical variances among families of *P. oocarpa*. * Means followed by the same letter in a column are not significantly different at the 0.05 probability level (Duncan's Multiple Range Test).

In mother trees, the following traits showed statistically significant differences: area, Feret´s diameter, and density of RD ($p < 0.05$). As for the genetic and environmental factors that contributed to the development of the anatomical traits (the components of variance), our results suggest that the variance among families in the progenies (attributable to the genetic factor) had a contribution to the total phenotypic variance that ranged from 0.00 ≥ σ^2_f % ≤ 16.21. However, the variance of the error (σ^2_e) was the one that had a more significant contribution to the total phenotypic variance for all the variables as it is usual for half-sib progeny tests (87.45 ≥ σ^2_e % ≤ 100). The coefficients of variation in progenies varied for all traits from 9.42 ≥ *CV %* ≤ 32.01 and in mother trees from 7.00 ≥ *CV %* ≤ 89.68. For more details, see **Table 2.2**.

Table 2.2 ANOVA by family for trees from a progeny trial of *P. oocarpa*.

Source of variation		**Juveniles**				**Adults**			
Variable	***Duct***	***d. f.***	***p***	***% F***	***% Error***	***d. f.***	***p***	***% F***	***% Error***
Area (mm^2)	AD	7	0.636	0.00	100.00	23	**0.000**	23.11	76.89
	RDX	7	0.356	0.00	100.00	23	**0.000**	46.84	53.16
	RDP	7	0.313	2.85	97.15	20	**0.000**	42.21	57.79
Diameter (mm)	AD (Feret)	7	0.616	0.00	100.00	23	**0.000**	17.54	82.46
	AD (Method A)	6	0.247	3.97	96.03	21	**0.048**	7.04	92.96
	AD (Method B)	7	0.646	0.00	100.00	19	**0.000**	43.49	56.51
	RDX	7	0.274	1.30	98.70	23	**0.000**	41.50	0.00
	RDP	7	0.453	0.59	99.41	20	**0.000**	45.98	0.00
Density (no./mm^2)	AD	7	**0.050**	11.79	88.21	.	.	99.01	0.00
	RDX	7	0.610	0.00	100.00	23	**0.000**	33.86	0.00
	RDP	7	0.349	2.34	97.66	22	**0.000**	29.65	0.00
Epithelial cells (per canal)	AD	7	0.084	12.55	87.45	11	0.057	56.47	43.53
	RDX	7	0.262	2.01	97.99	17	0.658	1.60	98.40
	RDP	7	0.262	0.00	100.00	16	0.063	9.66	90.34

AD = axial ducts, RDX = xylem radial ducts, RDP = phloem radial ducts, TD = traumatic ducts, d. f. = degrees of freedom, % Fam = family variance, % E = variance of error, * just showed the significant results

For the area and diameter of the AD, the differences between ages were significant. On the contrary, for the RD the differences were not significant. For the density, there were significant age differences only for RDP (**Table 2.3**).

Table 2.3 GLM analysis by age of juvenile (progenies) and adults (mother trees).

Source of variation		**Tree family**			**Tree age**		
Variable	**Type of canal**	**d. f.**	***p***	**% F**	**d. f.**	***p***	**% Error**
Area (mm^2)	AD	24	0.129	0	1	**0.005**	61
	RDX	24	**0.033**	78	1	0.921	22
	RDP	21	0.053	72	1	0.830	28
Diameter (mm)	AD	24	**0.050**	0	1	**0.001**	70
	RDX	24	**0.007**	86	1	0.336	13
	RDP	21	0.138	56	1	0.729	44
Density (no./mm^2)	AD	22	0.832	2	1	0.811	93
	RDX	24	0.557	0	1	0.092	58
	RDP	23	0.468	0	1	**0.019**	33

The narrow-sense heritability estimates (h^2_i) for the trait that showed variability among families were 0.35 (*SE*±0.11) and at family mean level (h^2_f) 0.53 (*SE*±0.32), the coefficient of genetic variation was 9.2, and finally the genetic gain was 13.30% with a selection index (*i*) of 1.4.

Variance between tissues, ages (mature and juvenile) and between tissues and ages. The difference in size and number of the resin ducts between mother trees and their young progenies and between different tissues are shown in **Fig. 2.2.** There were not AD in phloem tissue, just in xylem. On the other hand, the results also indicate that there were size differences between tissues and ages.

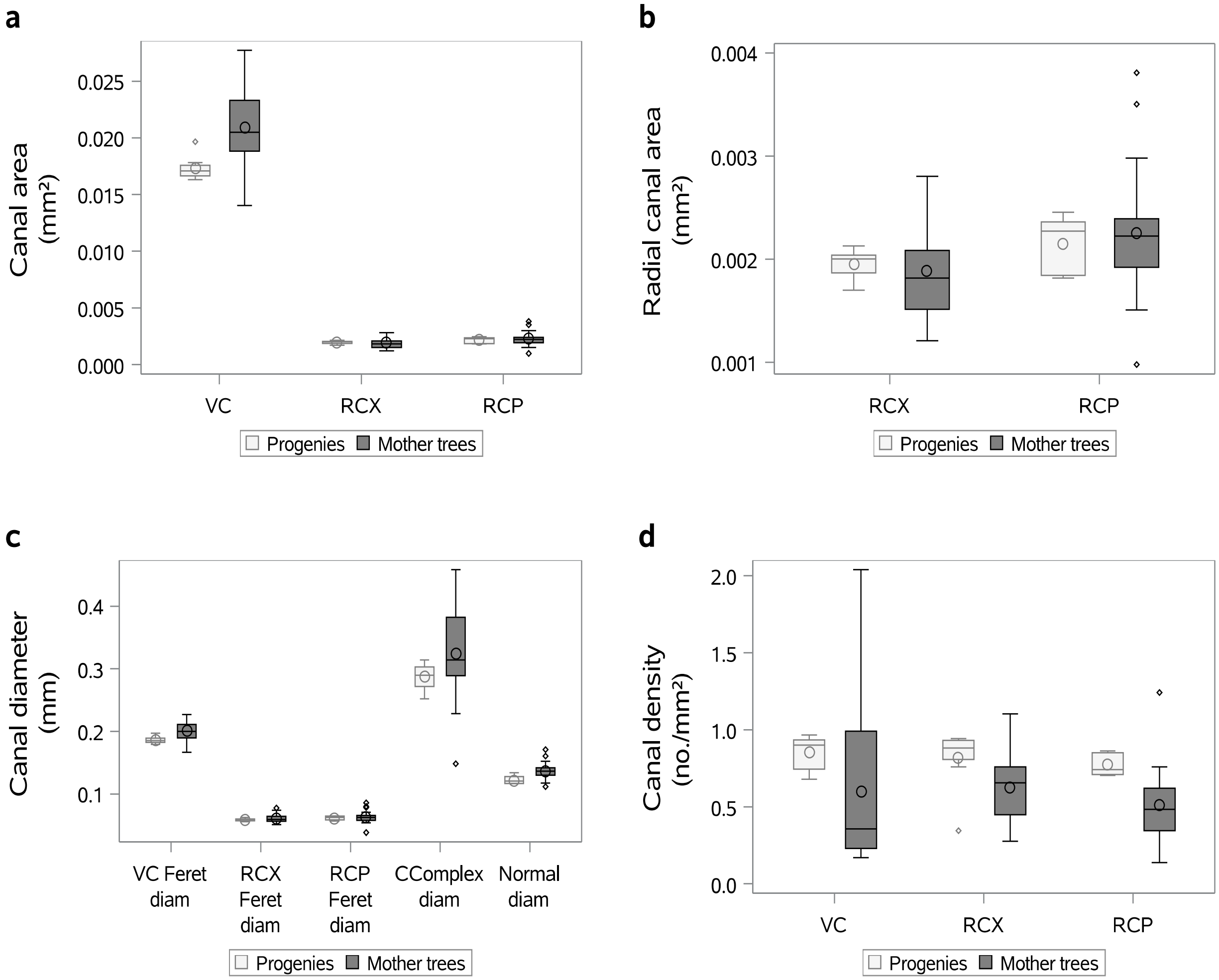

Fig. 2.2 Boxplots for mean data of the resin ducts by type and age.

Mean is the circle symbol, median the line, the upper and lower whiskers correspond to the maximum and minimum values, **a**, area of resin ducts; **b**, area of radial resin ducts; **c**, resin duct diameters; **d**, resin duct density. AD axial resin ducts, RDX radial resin ducts of the xylem, RDP radial resin ducts of phloem.

Xylem versus phloem

Size of ducts. The area of the ADs of the mother trees and young progenies was 89% - 91% larger than the area of the RD for both tissues. On average, the RDP of the mother trees were larger than the RDX (21%).

Density of ducts. The density of the AD was similar to the RDX density (-1%). However, on average there was a smaller number of AD than RDP, the difference ranged from 10% to 27%.

Young progenies vs. mother trees

Size of ducts. The area of ADs of the mother trees was, on average, 19% larger than the duct area of the progenies, indicating that the AD area increases with age. The RDX of the mother trees were smaller than those of the progenies (5%). However, the RDP of the mother trees were larger than in the progenies (15%).

Density of ducts. However, there was a smaller number of AD per mm^2 in the mother trees than in progenies (18.6%). In mother trees, there were fewer RDP and RDX per mm^2 compared to progenies. We differentiated between constitutive and induced resin duct formations and did not include tangential bands of axial resin ducts, which we considered formation (**Fig. 2.5a**).

Description of resin ducts of *Pinus oocarpa.* The wood of *P. oocarpa* had well-defined growth rings. Resin ducts and rays were visible to the naked eye (**Fig. 2.3a**, **b**). The growth-rings in mother trees were narrower (average 1.4 mm) and more uniform. The transition between late and earlywood was abrupt. In the young progenies, the tree-rings were wider (average 6.3 mm), and the transition from earlywood to latewood was less marked. ADs were in the earlywood, sometimes in latewood. ADs in adult and young progenies were mostly solitary (**Fig. 2.3**, **Fig. 2.4**). The epithelial cells of the ducts were surrounded by layers of axial parenchyma subsidiary cells (5-6 cells/duct) **(Table 2.3, Fig. 2.4a, b)**, which sometimes extended to the sides, forming an aliform tissue and having dark-staining contains (**Fig. 2.3c, d**). When the ducts were in pairs, the aliform tissue tended to converge. Three or more ducts in a row are traumatic resin ducts (**Fig. 2.3c**). On average, the aliform tissue of *P. oocarpa* was composed of 41 cells. The detailed descriptive statistics for the resin ducts are given in **Table 2.4**.

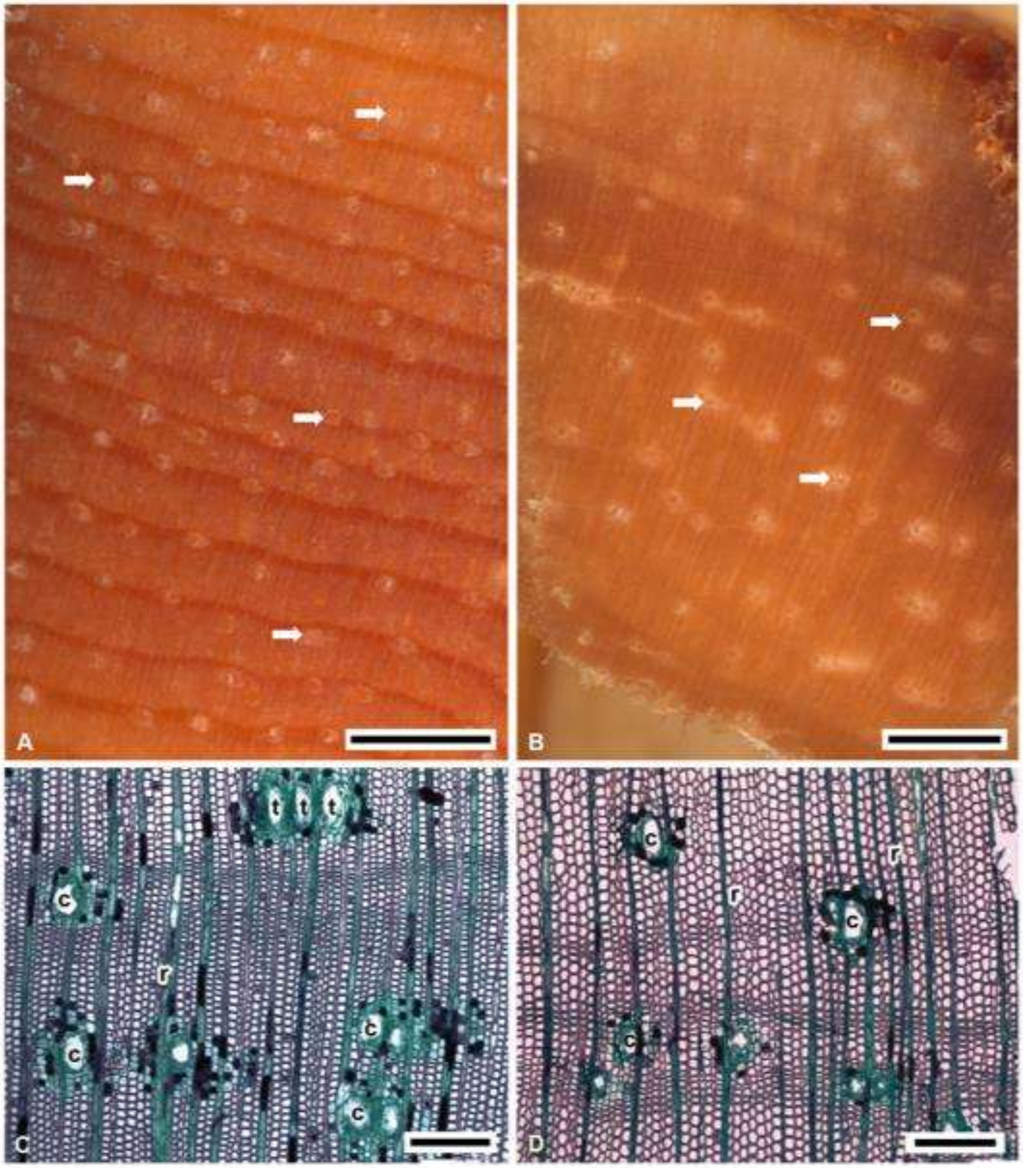

Fig. 2.3 *P. oocarpa* wood samples, visible growth rings, rays, axial and traumatic resin ducts.

a, adult tree; **b,** juvenile tree; **c**, adult wood transverse-section with normal and traumatic resin ducts; **d**, juvenile wood transverse-section with normal resin ducts. Scale bar is 2 mm in **a**, **b**; 300 µm in **c**; 50 µm in d. **c** = normal resin ducts, **r** = ray, **t** = traumatic resin duct.

Table 2.4 Descriptive statistics of anatomical characteristics (axial, radial ducts, epithelial cells, subsidiary cells) of *P. oocarpa* in progenies and mother trees.

Variable	Tree age	Type of duct	Mean	St. dev.	Min.	Max.	CV	Mode
Area (mm^2)	Juvenile	AD	0.0173	0.0038	0.0082	0.0282	21.95	.
		RDX	0.002	0.0004	0.0012	0.0033	19.63	.
		RDP	0.0021	0.0007	0.0011	0.0042	32.01	.
	Adult	AD	0.0211	0.0059	0.0087	0.0344	27.86	0.0168
		RDX	0.0019	0.0006	0.0006	0.004	31.6	0.0013
		RDP	0.0022	0.0009	0.0005	0.006	40.76	.
Diameter (mm)	Juvenile	AD (Feret)	0.186	0.209	0.117	0.229	11.22	.
		AD (method A)	0.122	0.175	0.088	0.17	14.35	0.121
		AD (method B)	0.289	0.646	0.05	0.466	22.36	.
		RDX	0.059	0.056	0.046	0.077	9.42	.
		RDP	0.062	0.091	0.047	0.09	14.78	.
	Adult	AD (Feret)	0.200	0.027	0.133	0.264	13.31	0.17
		AD (method A)	0.137	0.027	0.089	0.205	19.76	0.118
		AD (method B)	0.337	0.112	0.115	0.625	33.36	0.29
		RDX	0.061	0.01	0.039	0.093	16.2	0.063
		RDP	0.063	0.014	0.031	0.109	22.43	0.031
Density (no./mm^2)	Juvenile	AD	0.86	0.23	0.41	1.45	26.51	0.90
		RDX	0.87	0.27	0.21	1.72	30.86	0.55
		RDP	0.77	0.19	0.41	1.24	24.29	0.90
	Adult	AD	0.60	0.54	0.17	2.04	89.68	0.23
		RDX	0.61	0.35	0	1.72	57.6	0.69
		RDP	0.52	0.33	0	2.07	63.51	0.34
Epithelial cells (per ducts)	Juvenile	AD	5.4	0.74	4	7.5	12.93	5
		RDX	3.56	0.43	2.67	4.67	12.2	4
		RDP	3.6	0.45	3	5.5	12.53	3
	Adult	AD	5.8	0.57	5	7	9.85	6
		RDX	3.21	0.22	2.5	3.67	7	3.2
		RDP	3.22	0.33	2.67	4	10.16	3
Subsidiary cells (per duct)	Juvenile	AD	40.8	12.72	20	78.33	31.18	37

AD axial ducts, RDX radial ducts of xylem, RDP radial ducts of phloem, St. dv. standard deviation, CV coefficient of variation

RDs were found only within the multi-seriate rays of both secondary xylem and phloem (**Fig. 2.4c-d**). A single RD per ray was found (Richter et al. 2004); however, exceptionally, we found two RDs in the same ray (**Fig. 2.4e-h**), which is considered a unique trait (Panshin and de Zeeuw 1980). The number of epithelial cells in mother trees was three cells per duct in both xylem and phloem. In the progenies, an average of four epithelial cells per duct was found, four in the xylem (mode = 4) and three in the phloem (mode = 3), the area and density of RDs of mother trees in the xylem and phloem are given in **Table 2.4**.

In the wood and secondary phloem, other types of defense mechanisms were found, such as polyphenolic compounds and calcium oxalate crystals, both more abundant in some cells of the axial and radial secondary phloem parenchyma (**Fig. 2.5**).

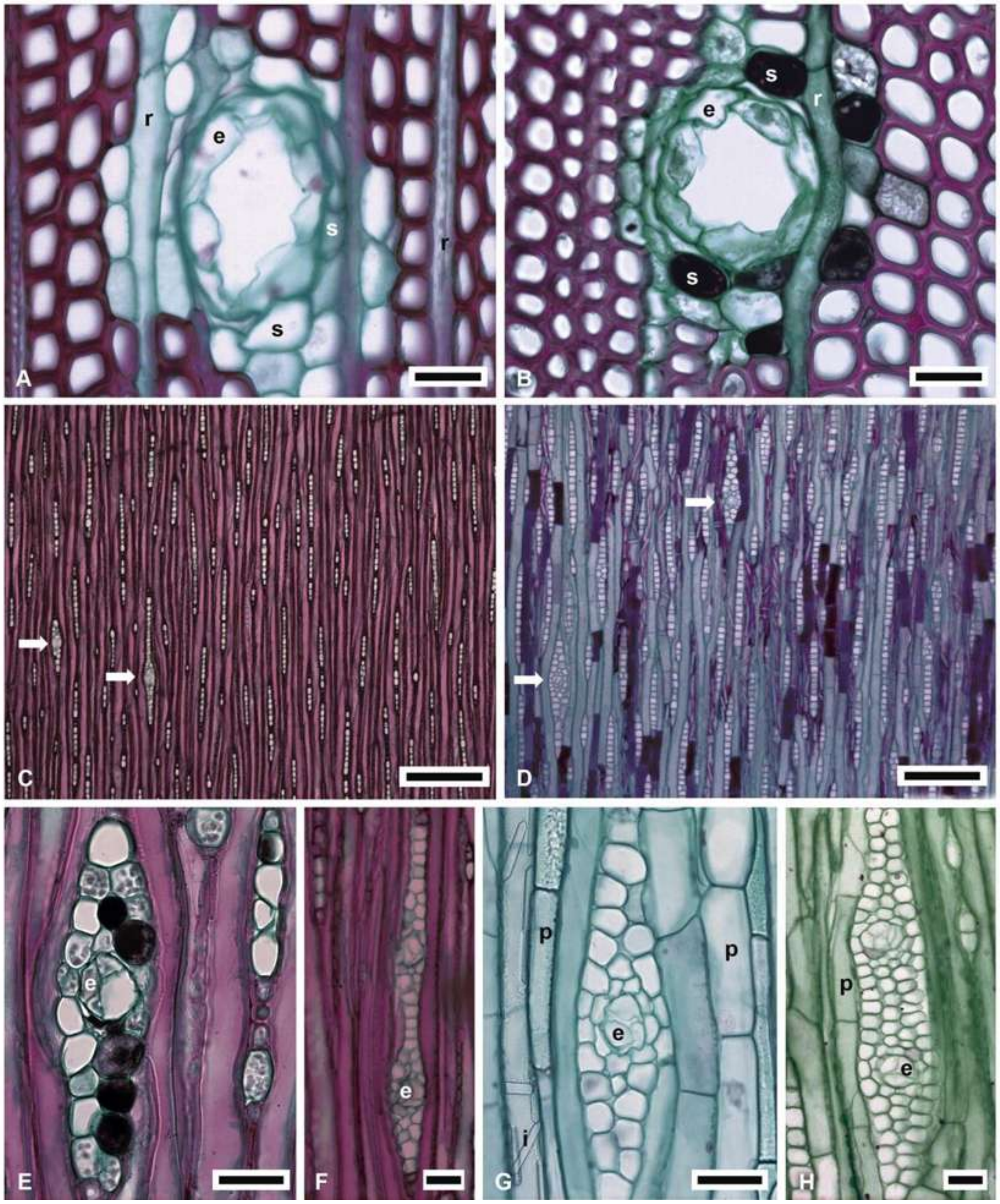

Fig. 2.4 Axial and radial resin ducts in *P. oocarpa*.

a, **b**, axial resin duct complex with epithelial cells and subsidiary cells, transverse sections; **c**-**h**, tangential sections; **c**, phloem with uniseriate and fusiform rays with a duct (arrow); **d**, wood with uniseriate and fusiform rays with a duct (arrow); **e**-**h**, detail of radial resin duct one or two per ray; **e**, **f**, wood; **g**, **h**, phloem. Scale bar is 50 µm **a**, **b**, **e**-**h**; 300 µm in **c**, **d**. **e** = epithelial cell, **i** = rhombohedron prism crystal, **r** = ray, **p** = parenchyma, **s** = subsidiary cells.

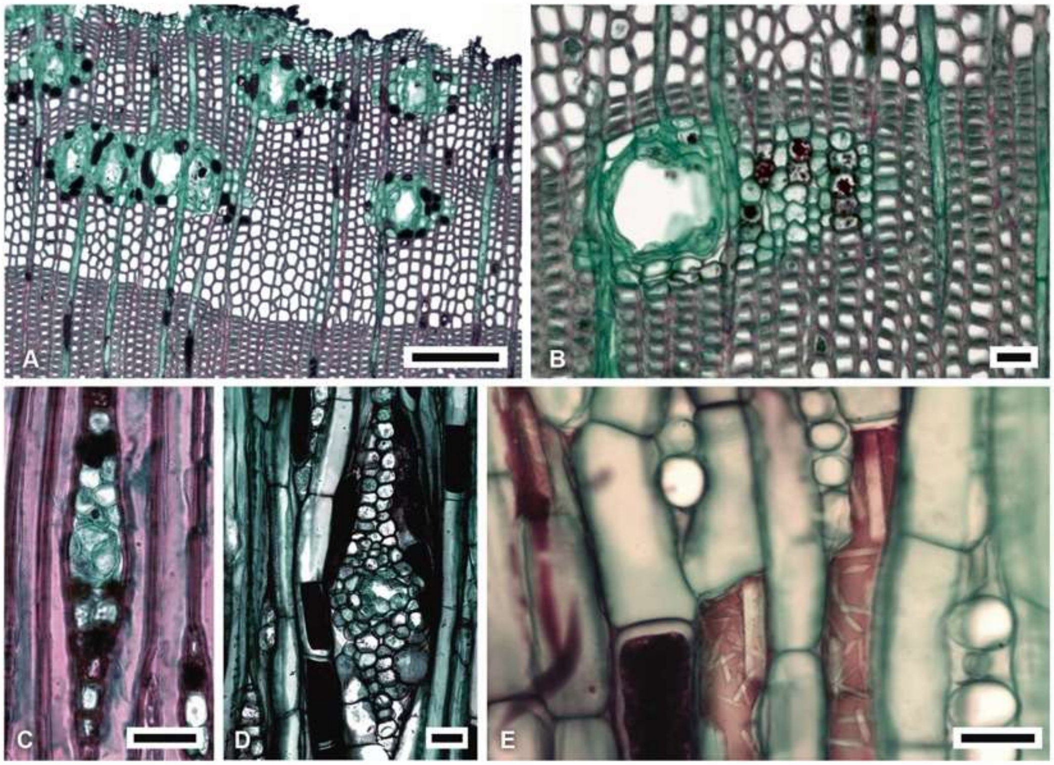

Fig. 2.5 Wood and secondary phloem in *P. oocarpa* with tannins and crystals.

a, tannins in subsidiary parenchyma cells of resin ducts and ray cells; **b**, detail of tannins in axial parenchyma; **c**, rays in wood with tannins; **d**, ray and axial parenchyma in p phloem with tannins; **e**, detail of parenchyma with tannins and rhombohedron prism crystals in phloem. Scale bar is 300 µm **a**; 50 µm in **b**-**e**.

2.4 Discussion

To our best knowledge, this study is the most thorough assessment of the resin duct system in *Pinus oocarpa*. The used technique allowed us to differentiate each type of duct (the constitutive from the induced), so we achieved high precision in our measurements. We found intraspecific variation in AD (constitutive) density, but no evidence of intraspecific genetic variation in size, diameter, or the number of epithelial cells. Of all the resin duct characteristics examined, only one trait presented enough variation to expect genetic improvement.

Genetic parameters and heritability. We found statistically significant differences among families for just one trait, AD density ($p < 0.05$), providing an opportunity for genetic improvement by selection (Bhat et al. 2016). A possible explanation is that specific differences are often less clearly expressed in wood anatomical features than morphological features (Longui et al. 2017) because morphology experience higher selection pressure (Herendeen and Miller 2000). Also, resin structure differentiation processes are slower and invest a larger number of carbon sources in comparison with chemical defense processes, which are rapid and less expensive (Bonello et al. 2006; Moreira et al. 2015). The fact that AD density is the only feature that expressed intraspecific variation seems in agreement with reports for *P. taeda* and *P. radiata* in clonal and full-sib trials (Westbrook et al. 2015; Peter 2018; Govina et al. 2020). Also, significant differences among provenances for AD density in branches of 10 years old *P. pinaster* were reported (Zas et al. 2015) in agreement with our results. For other conifers as *Picea abies*, all resin duct traits had wide genetic variation and high broad-sense heritabilities (H^2), with values between (0.28 and 0.82) (Rosner and Hannrup 2004).

Besides, the estimated narrow-sense heritability for AD density ($h^2_i = 0.35$) was moderate, similar to estimated heritabilities for another species such as *P. radiata* ($h^2_i = 0.33$; 2 yrs; Govina et al. 2020). The heritability at family mean level (h^2_f) was high, indicating that 53% of the phenotypic variation is explained by the family differences. This estimate was slightly higher to that obtained for *Picea abies* (19 years old) in clones and full-sib families for the same trait ($H^2 > 0.4$; Rosner and Hannrup 2004), and higher to that estimated for clones of *P. taeda* ($H^2 = 0.12 – 0.21$; Westbrook et al. 2015). The differences may reflect the fact that genetic parameter estimates from clonal trials are assumed to be more precise than that of the corresponding estimates from progeny trials (Rosner and Hannrup et al. 2004; Govina et al. 2020). As for the size of ducts, the lack of intraspecific variability seems to limit the possibility of improvement (of genetic gain), and therefore the response to selection will not be significant (Govina et al. 2020). Notably, pine species studied up today show that AD number (density) is the unique variable with enough variation for breeding (Westbrook et al. 2015; Peter 2018; Govina et al. 2020). Notably, pine species studied up today show that AD number (density) is the unique variable with enough variation for breeding (Westbrook et al. 2015; Peter 2018; Govina et al. 2020). Notably, pine species studied up today show that AD number (density) is the unique variable with enough variation for breeding (Westbrook et al. 2015; Peter 2018; Govina et al. 2020). We also found this pattern in *P. oocarpa*, as mentioned above, suggesting that probably this pattern is a genus trait, thus other species of *Pinus* need to be studied to support this assertion.

Variance between tissues, ages (mature and juvenile), and between tissues and ages. *Xylem vs. Phloem*. In the young progenies, no significant differences ($p = 0.06$) were found between the size of RDP and RDX. On the contrary, in the mother trees, the differences in size between RDP and RDX were significant ($p = 0.02$). As for the RD density, differences between phloem and xylem in the progenies were highly significant ($p = 0.004$). Our results differ with those reported in *P. taeda*, where no differences in radial duct density between tissues were detected (Blanche et al. 1992), but the average density of RDP ($0.30/\text{mm}^2$) was much lower than that found in *P. oocarpa*.

Young progenies vs. mother trees. Size of ducts. As for the differences of the resin duct features between the mother trees and the young progenies, the mean area of the ADs was larger in the mature trees. For the area of the RDX, the difference was minor. Conversely, regarding the RDP, the expressed variances in duct size between the two ages was larger. In this case, it could be a consequence of the cellular arrangement in the phloem; since, in the mature trees, there is more space for a cell to expand (Evert 2006). Therefore, it seems that the size of the RDP increases with age (Thomson and Sifton 1925), and larger ducts may enhance its resistance because small increases in resin duct lumen area can significantly increase resin flow (Schopmeyer et al. 1954). The larger area of the ducts in the phloem could be explained based on the optimal defense hypothesis (Cipollini et al. 2018) as a consequence of the differential allocation of plant defenses among plant parts (Erbilgin and Colgan 2012). The phloem is a more vulnerable tissue, because it is the first protective barrier against stem borers and the main target for wounding insects (Moreira et al. 2012).

Density of ducts. The density of ducts showed a different pattern than size. In mother trees, we found fewer AD, RDX and RDP per mm^2. This difference could be a matter of age because the number of RDX per unit of tangential surface and the growth of the rings seems to decrease with tree maturity (Reid and Watson 1966; Larson 1994, DeAngelis et al. 1986). According to Mergen and Echols (1955), in *P. elliottii* the number of RDX per mm^2 per ring at an early age (5 years old) was much higher than in adult trees. A similar trend was found in trees of *P. ponderosa* (Kane and Kolb, 2010) and *Picea abies* (Rosner and Hannrup 2004); similar to what we found for *P. oocarpa*. However, authors such as Takizawa et al. (1980) observed an opposite pattern (i.e. radial ducts increased form pith to bark) in *Larix leptolepis* Gord. Erbilgin and Colgan (2012) suggests differences in constitutive defenses between mature trees and juvenile trees. Since mature trees tend to have well developed preformed physical defenses (e.g., stone cell masses, periderm layers, and calcium oxalate crystals, and thus they rely to a lesser extent on their biochemical defenses in comparison with the juvenile trees (Erbilgin and Colgan 2012). Another possible explanation could be that young progenies are less tolerant of damage than mature plants (Haukioja and Koricheva 2000), so they need a more considerable amount of immediately available chemical defenses (preformed) as opposed to mature trees. The larger size of the trees allows them to have a higher capacity to induce its carbon-based secondary compounds (Moreira et al. 2016), that are produced or increased only after herbivore damage (Boege et al. 2005) and are less costly since they are produced only when needed

(Moreira et al. 2016). Consequently, with these mechanisms, we also found that the oxalate crystals, the primary function of which is to obstruct and delay the attacks of bark beetles (Hudgins et al. 2003), were larger in the mature trees (38%) than in the juvenile trees, in other words, the physical defenses were better developed in mature trees than in the young ones.

Our results also evidence that the resin duct differences between ages were, in some cases, statistically significant, but different patterns were observed depending on the tissue and type of canal. The traits that changed most with age were in xylem, the area and diameter of the AD and in phloem density of the RD. The size and density of the RDX changed the least with tree development.

Description and variability in resin features. In both, mother trees and young progenies, axial, radial, and traumatic resin ducts had a general morphology as was described for other pine species (e.g., Richter et al. 2004). A distinctive feature was the lack of axial resin ducts in the secondary phloem as in other species such as *P. radiata* D. Don, *P. halapensis*, *P. tabulaeformis* Carr., *P. nigra*, *P. menziesii*, *P. cembroides*, *P. oaxacana*, and *P. densiflora* (Chattaway 1951; Werker and Fahn 1969; Patel 1975; Zenghai et al. 1987; Yáñez-Espinosa and Terrazas, 1998; Zhang et al. 2008). Though, this differs from what was found in *P. maximartinezii* (Yáñez-Espinosa and Terrazas 1998), where both axial and radial resin ducts were present in secondary phloem. The microstructure of the AD complex (group of epithelial cells, intercellular spaces, and subsidiary cells) (Wiedenhoeft and Miller 2002) was very distinctive for the *P. oocarpa* individuals already from an early age. Most of the AD were solitary, as noted for *P. cembra*, *P. longeava*, and *P. radiata* (Grosser 1977; Baas 1982; Richter et al. 2004, Thomas and Collings 2017). However, occasional anastomoses of radial and axial resin ducts in the xylem were found in the tangential sections. This trait was also reported in *P. contorta* (Reid and Watson 1966), *P. densiflora* (Zhang et al. 2008), P*. halapensis* (Werker and Fahn 1969), *P. taeda* (LaPasha and Wheeler 1990), various pine species from southern USA (Howard and Manwiller 1969), and *P. elliottii* where evidence that this trait is related to resin yield was given (Neis et al. 2019b).

Comparison of resin duct features with previous studies is difficult, because ages are not always reported, and the influence of climate, soil, and trials are factors to consider. Overall, the most studied ducts have been the AD, followed by the RDX, and the least the RDP. The mean size of the AD of progenies of *P. oocarpa* was identical to that of full-sib families and clones of *P. radiata* (0.017 mm^2; Govina et al. 2020). We must consider that the trees were two years old and that the aim of their study was to improve *P. radiata* wood value. Since for some species resin ducts are considered as defects in the wood (Ananías et al. 2010), however the resin yield of *P. radiata* is low to be considered economically viable for commercial tapping (da Silva Rodrigues-Corrêa et al. 2013). We found differences in comparison with other studies and species too. The ADs size of the mother trees of *P. oocarpa* was smaller than in *P. elliottii* (Neis et al. 2019b). However, it was similar to *P. contorta* (117 years old) and higher than in *P. flexilis* (253 years old, Ferrenberg et al. 2014). Nevertheless, they were much smaller than in *P. pinaster* (Rodríguez-García et al. 2014), because measuring techniques (area included the adjacent parenchyma cells) were different.

The diameter of the ADs was three times larger than that of the RDXs, which agrees with previous report by Koch (1972). Consistently, the number of epithelial cells (5-7 cells) was higher in the ADs than in the RDXs, and it was similar to those reported for *P. radiata* (5-6 cells) (Cown 2010), slightly lower than those found in *P. pinea* (Wooding and Northcote 1965), and *P. caribaea* var. hondurensis (6-8 cells) (Boschiero and Tomazzello-Filho 2012).

According to the classification proposed by the IAWA Committee (Richter et al. 2004), in the *P. oocarpa* progenies, the diameter of the ADs (method A) is medium. Since the average was in the range for the IAWA group (0.100-0.170 mm) as medium, which is considered representative of the Pinaceae. However, if we consider the Feret´s diameter for both mother trees and progenies, *P. oocarpa* would be classified within the group of species with large ADs (0.170-0.300 mm). Several studies have previously assessed the density of ADs in various *Pinus* species (**see Supplementary Table 2.2**). The mean AD density in the mother trees of *P. oocarpa* was similar to that of species such as *P. pinaster* and *P. canariensis* (Rodríguez-García et al. 2014; Esteban et al. 2005).

The RDXs fulfill two main functions in the overall defense system, they synthesize the new resin and supply it to the attack/injury sites (DeAngelis et al. 1986), helping to strengthen the defenses (Zhang et al. 2008). In agreement with what was found in *P. halapensis* (Werker and Fahn 1969) and *P. densiflora* (Zhang et al. 2008) in *P. oocarpa*, the subsidiary cells were found on one, both or none of the sides of the duct (Fig. 2.2). The Feret´s diameter of the RDX of the young progenies and the mother trees (0.059 – 0.061 mm) was similar to the average found in various species, which are also commercially exploited for resin such as *P. caribaea* var. hondurensis, and *P. pringlei* Shaw (**Supplementary Table 2.3**). *P. caribaea* is a high yielding species (Coppen and Hone 1995) and *P. pringlei*, a Mexican endemic species, is one of the most resin yielding species in Mexico (Gutiérrez-Jarquín 1977) and probably the size of the RDX contributes in the three species to the production. Some authors have reported that typically there is a larger number of RDX than AD in normal wood as for *Pseudotsuga menziesii* and *Larix decidua* (Larson 2004). Nevertheless, we found in *P. oocarpa* a different pattern, in general ADs were more numerous than the RD for both ages and tissues, with one exception, the RDX in the progenies where we found no difference in the mean RDX density in comparison with the mean AD density. We do not know if this different pattern is a genus trait. Thus, further studies in the normal wood of other *Pinus* species are needed.

2.5 Conclusion

We found in *P. oocarpa*, the typical defensive traits of the stems of the pines via resin ducts. The storage structures of the defensive system of *P. oocarpa* showed age-dependent changes and differences in mean values. Axial and radial resin ducts showed variances in their size and quantity. The mean size of the AD increased with age. The RD maintained more uniformity from an early age in both tissues. However, the mean density of all the resin ducts types became lower with age. As for the constitutive resin features, the observed intraspecific variability of the density of the AD suggests that the density of resin ducts seems to be the trait that is less influenced by environmental factors. Thus, AD density is likely the most susceptible to be explored for a tree breeding program, as all the progenies are growing under same environment and are of the same age. The trait that showed the least intraspecific variation was the size of the resin ducts. However, the results from this study are from a small sample (eight families, one site), but they provide a good starting point for analyses involving larger data sets, although there also exists evidence that these anatomical features are very stable through environments. The data contribute to our understanding of the intraspecific variation of the resin duct system of the secondary body of *P. oocarpa*. They could also help to develop better management strategies in *P. oocarpa* forests harvested for resin, as there is an increasing demand for sustainable forest resources.

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Supplementary Table 2.1 List of representative studies for stem resin duct features This list is not intended to be comprehensive, but rather to illustrate the range of previously conducted work.

Subgenus	**Subsection**	***Species***	**No.**	**References**
Trifoliae	Australes	*P. caribaea*	2	Gerry 1935, Boschiero and Tomazello-Filho 2012
Trifoliae	Australes	*P. echinata*	1	Hodges et al. 1981
Trifoliae	Australes	*P. elliottii*	9	Hobert 1932, Schopmeyer et al. 1953, Mergen and Echols 1955, Kibblewhite et al. 1971, Kibblewhite and Thompson 1973, Hodges et al. 1981, Wu and Hu 1997, Nagy et al. 2000, Neis et al. 2019b
Trifoliae	Australes	*P. glabra*	1	Howard and Manwiller 1969
Trifoliae	Australes	*P. herrerae*	2	Olvera-Coronel 1981, Pérez-Olvera and Dávalos-Sotelo 2016
Trifoliae	Australes	*P. leiophylla*	1	Pérez-Olvera and Dávalos-Sotelo 2016
Trifoliae	Australes	*P. palustris*	1	Hodges et al. 1981
Trifoliae	Australes	*P. pringlei*	1	Olvera-Coronel 1985
Trifoliae	Australes	*P. pungens*	1	Howard and Manwiller 1969
Trifoliae	Australes	*P. radiata*	9	Chattaway 1951, Patel 1971, Zhenghai and Ruching 1987, Yang et al. 2007, Ananías et al. 2010, Cown et al. 2010, Chen et al. 2017, Thomas and Collings 2017, Govina et al. 2020
Trifoliae	Australes	*P. rigida*	1	Lin et al. 2002
Trifoliae	Australes	*P. taeda*	13	Howard and Manwiller 1969, Hodges et al. 1981, DeAngelis 1986, La Pasha and Wheeler 1990, Blanche et al. 1992, Lombardero et al. 2000, Lin et al. 2002, Strom et al. 2002, Tisdale et al. 2003, Westbrook et al. 2013, Eckert et al. 2013, Westbrook et al. 2015, Turner et al. 2019
Trifoliae	Australes	*P. teocote*	2	Olvera-Coronel 1981, Pérez-Olvera and Dávalos-Sotelo 2016
Trifoliae	Contortae	*P. clausa*	1	Howard and Manwiller 1969
Trifoliae	Contortae	*P. contorta*	7	Reid and Watson 1966, White and Nilsson 1983, Wiedenhoeft et al. 2002, Wiedenhoeft et al. 2003, Freeden et al. 2004, Ferrenberg et al. 2014, Zhao and Erbilgin 2019, Mason et al. 2019
Trifoliae	Ponderosae	*P. arizonica*	2	Olvera-Coronel 1985, Pérez-Olvera and Dávalos-Sotelo 2016
Trifoliae	Ponderosae	*P. cooperi*	1	Pérez-Olvera and Dávalos-Sotelo 2016
Trifoliae	Ponderosae	*P. douglasiana*	1	Olvera-Coronel 1985
Trifoliae	Ponderosae	*P. durangensis*	2	Pérez-Olvera and Dávalos-Sotelo 2016
Trifoliae	Ponderosae	*P. michoacana*	1	Olvera-Coronel 1981
Trifoliae	Ponderosae	*P. montezumae*	1	Olvera-Coronel 1985
Trifoliae	Ponderosae	*P. ponderosa*	6	Smith 1968, Wiedenhoeft et al. 2002, Wiedenhoeft et al. 2003, Kane and Kolb 2010, Hood and Sala 2015, Hood et al. 2015, Pinnell 2016 (thesis)

Trifoliae	Ponderosae	*P. pseudostrobus*	1	Olvera-Coronel 1981
Trifoliae	Ponderosae	*P. tenuifolia*	1	Olvera-Coronel 1981
Pinus	Pinaster	*P. canariensis*	3	Climent et al. 1993, Esteban et al. 2005, Chano et al. 2015
Pinus	Pinaster	*P. halapensis*	5	Werker and Fahn 1969, Fahn and Zamski 1970, Zamski 1972a, Zamski 1972b, Fahn and Benayoun 1976
Pinus	Pinaster	*P. pinaster*	11	Lin et al. 2002, Moreira et al. 2008, Ballesteros et al. 2010, Carde 2014, Rodríguez-García et al. 2014, Moreira et al. 2015, Rodríguez-García et al. 2015, Zas et al. 2015, Rodríguez-García et al. 2016, Di Mateo and Voltas 2016, Vázquez-González et al. 2019
Pinus	Pinaster	*P. pinea*	2	Wooding and Northcote 1965, Lev-Yadun, 2002
Pinus	Pinaster	*P. roxbourghii*	1	Lin et al. 2002
Pinus	Pinaster	*P. leucodermis*	1	Saracino et al. 2017
Pinus	Pinus	*P. densiflora*	2	Zhang et al. 2008
Pinus	Pinus	*P. massoniana*	5	Wu and Hu 1997, Lin et al. 2002, Li et al. 2004, Li et al. 2009, Liu et al. 2013
Pinus	Pinus	*P. nigra*	4	Patel 1971
Pinus	Pinus	*P. sylvestris*	9	Chudnyi 1974, Wu and Muller 1999, Bosshard and Hug 1980, Lin et al. 2002, Rigling et al. 2003, Kilpeläinen et al. 2007, Martín et al. 2010, Moreira et al. 2012, Moreira et al. 2015
Pinus	Pinus	*P. tabulaeformis*	4	Wu et al. 1987, Hong 1990, Wu and Hu 1997, Lin et al. 2002
Pinus	Pinus	*P. tumberghii*	3	Ishida and Hogetsu 1997, Wu and Hu 1997, Lin et al. 2002
Pinus	Pinus	*P. taiwanensis*	2	Wu and Hu 1997, Lin et al. 2002
Pinus	Pinus	*P. yunnanensis*	2	Wu and Hu 1997, Lin et al. 2002
Pinus	Pinus	*P. finlaysoniana*	1	Wu and Hu 1997
Strobus	Gerardiana	*P. bungeana*	2	Wu and Hu 1997, Lin et al. 2002
Strobus	Strobus	*P. armandii*	2	Wu and Hu 1997, Lin et al. 2002
Strobus	Strobus	*P. ayacahuite*	1	Olvera-Coronel 1985
Strobus	Strobus	*P. flexilis*	1	Olvera-Coronel 1981
Strobus	Strobus	*P. koraiensis*	2	Wu and Hu 1997, Lin et al. 2002
Strobus	Strobus	*P. monticola*	2	Hudgins et al. 2003, Hudgins et al. 2005
Strobus	Strobus	*P. parviflora*	1	Lin et al. 2002

Source of information on genus, subgenus, subsection: Tree of Life Web Project
http://tolweb.org/tree/

Supplementary Table 2.2 Area, diameter, and density of axial xylem resin ducts (area – mm^2; diameter = mm; density = no./mm^2, age=tree age)

Trait	***Species***	***Mean***	***Age***	***Reference***
Area	*P. contorta*	0.018 – 0.020	117	Ferrenberg et al. 2014
	P. elliottii	0.024 – 0.044	18	Neis et al. 2019b
	P. flexilis	0.014 – 0.017	253	Ferrenberg et al. 2014
	P. pinaster	0.037 – 0.041	75	Rodríguez-García et al. 2014
	P. pinaster	0.017 (clones) 0.017 (full-siblings)	2	Govina et al. 2020
Diameter	*P. canariensis*	0.22 (0.17-0.29)	x	Esteban et al. 2005
	P. caribaea	0.14	x	Boschiero & Tomazzello Filho 2012
	P. contorta	0.06 – 0.105	75,30	Reid & Watson 1966
	P. oocarpa	0.19	x	Olvera-Coronel 1985
	P. pringlei	0.22	x	Olvera-Coronel 1985
	P. ponderosa	0.23 – 0.36	5,10,20	Kane & Kolb, 2010
	P. arizonica, *P. cooperi,* *P. durangensis,* *P. herrerae* *P. leiophylla,* *P. teocote*	0.072–0.127	x	Pérez-Olvera & Dávalos-Sotelo 2016
Density	*P. canariensis*	0.55 (0.25 – 0.60)	x	Esteban et al. 2005
	P. caribaea	0.34	14 - 19	Hoberd 1932
	P. caribaea var. hondurensis	0.39 (0.27 – 0.66)	43	Boschiero & Tomazzello Filho 2012
	P. elliottii	0.13 – 0.30	18	Neis et al. 2019b
	P. pinaster	0.5 – 1.5	14	Ananías et al. 2010
	P. pinaster	0.59 (0.57 – 0.62)	75	Rodríguez-García et al. 2014
	P. ponderosa	0.65 – 0.80	5,10,20	Kane & Kolb 2010
	P. radiata	0.69 (clones) 0.90 (full-siblings)	2	Govina et al. 2020
	P. armandi, *P. bungeana,* *P. densiflora,* *P. elliottii,* *P. finlaysoniana,* *P. koraiensis,* *P. massoniana,* *P. tabulaeformis,* *P. taiwanensis,* *P. thurnbergii,* *P. yunnanensis*	4 - 5	x	Wu & Hu 1997
	P. arizonica, *P. cooperi,* *P. durangensis,* *P. herrerae*	1	x	Pérez-Olvera & Dávalos-Sotelo 2016

	P. leiophylla, *P. teocote*			
	P. arizonica, *P. ayacahuite,* *P. douglasiana,* *P. montezumae,* *P. oocarpa,* *P. pringlei,* *P. teocote*	1-2	x	Olvera-Coronel 1985
	P. durangensis, *P. flexilis,* *P. herrerae,* *P. michoacana,* *P. pseudostrobus,* *P. reflexa,* *P. tenuifolia*	1-2	x	Olvera-Coronel 1981

Supplementary Table 2.3 Xylem radial ducts in *P. oocarpa*.

Trait	**Species**	**Mean**	***Age***	**Reference**
Area	*P. pinaster*	0.012- 0.013	75	Rodríguez-García et al. 2014
Density	*P. caribaea*	0.32	43	Boschiero& Tomazzello-Filho 2012
	P. densiflora	0.76	x	Zhang et al. 2008
	P. pinaster	0.72 (0.65 – 0.79)	75	Rodríguez-García et al. 2014
	P. taeda L.	0.36	30	Blanche et al. 1992
	P. armandi, *P. bungeana,* *P. densiflora,* *P. finlaysoniana,* *P. koraiensis,* *P. massoniana,* *P. tabulaeformis,* *P. taiwanensis,* *P. thurnbergii,* *P. yunnanensis*	0.5 – 2.0	x	Wu and Hu 1997
Diameter	*P. canariensis*	0.036	x	Esteban et al. 2005 (method A)
	P. caribaea	0.058	43	Boschiero & Tomazzello-Filho 2012
	P. echinate	0.049	59	Hodge et al. 1981
	P. elliottii	0.055	32	Hodge et al. 1981
	P. halapensis	0.051	7	Esteban et al. 2010
	P. oocarpa	0.055	x	Olvera-Coronel 1985
	P. palustris	0.058	46	Hodge et al. 1981
	P. pringlei	0.064	x	Olvera-Coronel 1985
	P. taeda	0.055	44	Hodge et al. 1981
	P. arizonica, *P. cooperi,* *P. durangensis,* *P. herrerae*, *P. leiophylla,* *P. teocote*	0.026-0.052	x	Pérez-Olvera & Dávalos-Sotelo 2016

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Capítulo 3 : Variación genética del diámetro de Feret de canales traumáticos y su asociación con la producción de resina de familias de medios hermanos de árboles seleccionados por su alta productividad de resina en Michoacán, México

Feret´s diameter of traumatic ducts varies genetically and is associated to resin yield of *P. oocarpa* half-sib progenies of highly resin productive selected trees in Michoacán, México

Resumen

P. oocarpa Schiede ex Schltdl. es el pino que más se resina en México. México ocupa el quinto lugar en el mundo en producción de resina, y es uno de sus recursos forestales no maderables más importantes. A pesar de su relevancia, no existe un programa de mejoramiento genético en el país que tenga como objetivo incrementar la producción de resina por unidad y tiempo para así poder mejorar la capacidad productiva de las especies nativas que se resinan a nivel comercial. Por lo tanto, el presente trabajo tuvo como objetivo estimar el grado de control genético del rendimiento de resina, del crecimiento (volumen de fuste), y así evaluar los caracteres anatómicos y las asociaciones entre cada uno de los rasgos a edad temprana en un ensayo de progenies de 27 familias de medios hermanos de P. oocarpa. La evaluación del rendimiento de la resina, el volumen y los rasgos anatómicos mostraron diferencias significativas entre las familias para casi todos los rasgos, excepto para el área total de los tres tipos de canales. Los resultados sugieren que la selección temprana de familias superiores de P. oocarpa parece ser factible con base en caracteres anatómicos (diámetros de Feret de canales traumáticos y/o la densidad de canales axiales). En general, el coeficiente de variación genética fue el más alto para el rendimiento de resina. La producción de resina tuvo una alta correlación genética con DAP ($r_g = 0.65$)*. Las estimaciones de heredabilidad fueron mayores para el volumen de producción de resina* ($h^2_i = 0.12 - 0.20$; $h^2_f = 0.44 - 0.59$) y menores para el volumen de madera ($h^2_i = 0.08 - 0.12$; $h^2_f = 0.29 - 0.46$), excepto para el tercer año del ensayo. Se encontró que las familias con canales axiales de xilema más grandes, pero con una menor densidad por mm^2 parecen ser más productivas, y que también es recomendable tomar en cuenta las defensas en respuesta a factores externos (es decir, la capacidad de respuesta del árbol) a la hora de seleccionar árboles para programas de mejoramiento genético.

Palabras clave: canales resiníferos, PFNM, resinación, sistema de defensa, producción de resina

Abstract

P. oocarpa is the most widely tapped pine tree in Mexico. Mexico ranks 5th in resin production in the world and is one of its most important non-timber forest resources. Despite its relevance, there is no genetic improvement program in the country aiming to improve the amount of resin production to increase the competitiveness of Mexican native species with resin potential. Therefore, the present work aimed to evaluate the degree of genetic control of resin yield, growth, anatomic traits, and the associations among them at an early age in a progeny trial of 30 half-sib families of *P. oocarpa* Schiede ex Schltdl., from selected mother trees, based on their yield production. Assessment for resin yield, stem volume, and anatomic traits showed significant differences among families for almost all traits except for the total duct area. Results suggest that the early selection of superior *P. oocarpa* families for resin yield, based on anatomic traits (Feret´s diameter of traumatic ducts and/or density of axial ducts), appears to be feasible. In general (for mother trees and progenies), the genetic variation among families was the highest for resin yield. Also, resin yield had a high genetic correlation with DBH (r_g = 0.65). Thus, estimates of heritability were higher for resin yield (h^2_i = 0.12 to 0.20; h^2_f = 0.44 to 0.59) and lower for volume (h^2_i = 0.08 to 0.12; h^2_f = 0.29 to 0.46) except for year three. We observed that half-sib families with larger but fewer axial ducts were more productive for resin yield. The ability to increase defenses in response to external challenges is also necessary to consider when selecting trees for breeding.

Keywords: NWFP, tapping, defense system, resin ducts, resin yield

3.1 Introduction

Coniferous trees of the pine family 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 types of defenses: 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) is an essential part of its defense mechanism. *Pinus* is the most diverse genus in the Pinaceae family (Langenheim 2003). In Mexico´s forests, there are found four genera (*Abies, Picea, Pinus*, and *Pseudotsuga*) of this family. These genera group 61 species, and *Pinus* (44) is the most diverse in species number (Gernandt and de la Rosa-Pérez 2014; Quiroz-Carranza and Magaña-Alejandro 2015). The pine species that are considered as suitable for commercial tapping in Mexico are *P. oocarpa*, *P. leiophylla*, *P. lawsonii*, *P. herrerai*, *P. tenuifolia*, *P. montezumae*, *P. ponderosa* and *P. pringlei* (Romahn-de la Vega 1982; Arias-Toledo and Chávez-López 2006; Quiroz-Carranza and Magaña-Alejandro 2015). Since Neandertal times humankind has used these secondary metabolites for its benefit (Degano et al. 2019), 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). Currently, the resin is a valuable terpene that is a natural source of raw material for various industries (chemical, pharmaceutical, perfume, food, cosmetics, biofuels, and fine chemicals). Its terpenoids have outstanding structural diversity and multiple chemical and physical properties (Zerbe and Bohlmann 2014). By the process of steam distillation, two fractions are obtained (rosin and turpentine), which are used for the manufacture of various products of daily and industrial use (Delgado-Macías 2015).

In Mexico, commercial exploitation of the resin 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, resin tapping is one of the most important non-timber forest resources, and the municipality of Ario is the main resin-yielding region of *P. oocarpa* (Francisco-Arriaga et al. 2011). 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). So far, all seedlings used for afforestation have been grown from seed material of mostly unknown origin and without any genetic improvement.

Therefore, this study aims to assess morphological and anatomical traits that are related to resin yield and its possible genetic control in *P. oocarpa* Schiede ex Schltdl. *P. 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).

Several studies indicate that the anatomical variables (frequency of the radial resin ducts, the total volume of ducts) and the morphological variables (the diameter and the percentage of the living crown) are related to the amount of resin production (Rodríguez-García et al. 2014; Neis et al. 2019). These traits could become useful indicators for the selection of superior trees in resin production in breeding programs (Rodríguez-García et al. 2014; Neis et al. 2019). Other studies suggest that the defense mechanisms of the Pinaceae (resin production, flow, composition) are heritable genetic characteristics and that through various genetic improvement techniques could increase the flow of resin from 1.5 to 2.4 times in a generation (Westbrook et al. 2013). In clones of *P. taeda* L., Westbrook et al. (2015) found that the number of axial resin ducts was also an inheritable characteristic (h^2 ~ 0.12 to 0.21). They also observed that there is a positive genetic correlation between the number of ducts and the growth of the xylem (r_g ~ 0.32 to 0.72) and the resin flow (r_g ~ 0.15 to 0.51), which suggests that *P. oocarpa* could have similar characteristics.

On the other hand, other studies reported that the yield of 3-year-old progenies of *P. elliottii* (early age) obtained by microchipping, is associated with the performance of their parents (r = 0.90). Microchipping consists of making small incisions in the tree to estimate resin yield at an experimental level (Squillace and Gansel 1968). These authors recommended using this microchipping technique for the early selection of highly productive genotypes. In *Pinus elliottii*, in Brazil, they estimated a high and significant correlation (*r* > 0.77) for resin yield among different ages (Romanelli and Sebben 2004). These findings confirm that there is a possibility of success in early selection (Gurgel-Garrido et al. 1994).

So, if a good correlation could be found between some of the assessed traits with resin yield, a higher number of high-yielding trees could be selected in less time. Also, it would mean 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, the research questions are a) How is resin yield correlated (genotypically and phenotypically) with quantitative/morphological traits (diameter, height, and volume) and its anatomical traits (resin ducts) in high yielding trees of *P. oocarpa* and their progenies?; b) Can the selection cycles of superior genotypes in resin production be shortened? In turn, we hypothesized first that resin yield, quantitative/morphological traits (volume), and anatomical characteristics (total area of resin ducts and area of traumatic ducts) significantly vary between families of half-siblings of *P. oocarpa* and thus they are under genetic control. Second, that volume of resin yield, morphological, and anatomical traits of *P. oocarpa* progenies could be related and become an index for the selection of superior genotypes in resin production at an early age.

3.2 Materials and methods

Plant material. The study material consisted of 30 open-pollinated families and 30 selected plus trees. All plus trees are in the Ejido San José de Cañas, Ario´s municipality, state of Michoacán de Ocampo, central-west México, in an area of approximately 1,007 hectares (**Fig. 3.1**). The trees are the property of local resin tappers and were considered by them as high yielding trees (Reyes-Ramos et al. 2019). Seeds were collected from these trees, seedlings were grown, and a progeny test was established in 2011. Because of the mortality of some seedlings, 27 families were eventually retained in the progeny test. The families are distributed in a randomized complete block design with 3x3 m between each tree. The experimental design consisted of 10 completely randomized blocks with three repetitions.

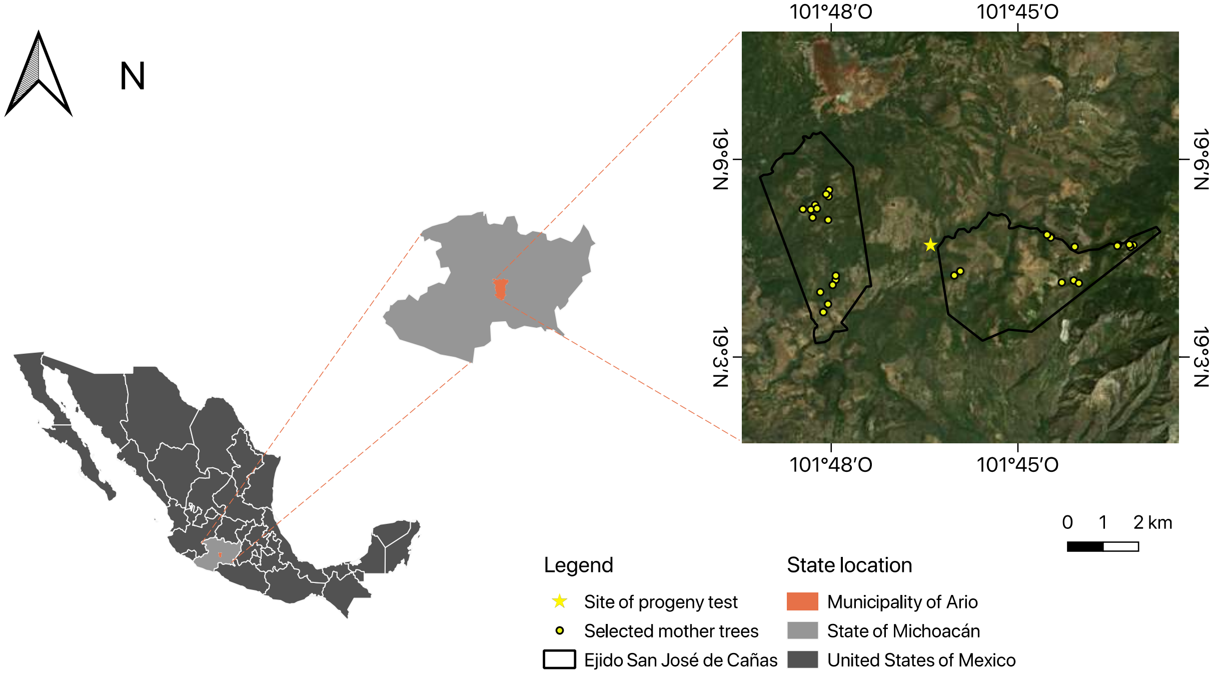


Fig. 3.1 Location of the progeny trial (yellow star) and trees selected as phenotypically superior in resin yield (yellow circles) of *P. oocarpa* in the municipality of Ario, state Michoacán, Mexico.

Resin yield assessment. We quantified the resin yield capacity of the half-sib families established in the progeny trial by the experimental method called microchipping. The technique allowed us to shorten the assessment period of the selected families (Gurgel-Garrido et al. 1999), and to evaluate resin yield at an early age on trees with small diameters without causing significant damage (Schopmeyer et al. 1954; Squillace and Gansel 1968; Alfaro 1995). At the time the first microchipping was conducted (year 3), the pines had a mean height of 2.2±0.03 m and mean stem diameter at breast height of 2.70±0.05 cm. 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 (Gonçalves-de Oliveira 1987). The acid worked as a stimulant to obtain the maximum yield and increase the duration of the flow (Clements 1974; Parham 1976). Then, 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 installation. The test was assessed at age three (RESp3), four (RESp4), five (RESp5), and six (RESp6) in April-May, when the harvesting season for resin is at its highest peak. During these months, which is the dry season in the area (1.8 months), water deficit is the largest, and the temperature is the highest. Also, soil water is depleted rapidly, and water storage reaches its minimum for the year, which is believed to enhance resin yield (Lorio 1994).

The resin yield of the parent trees (mother trees) was harvested twice. The first measurement was done weekly for a period of 5 months (January to May) in 2011 (variable coded as RESm1), and details are described in (Reyes-Ramos et al. 2019). The tapping was done by a local forest dweller using the traditional technique in Mexico; the Hugues method adapted to Mexico (Coppen and Hone 1995). The second evaluation was carried out by an adaptation of the borehole technique (Lekha and Sharma 2005), and the crude resin was harvested for 48 hours in 2017 (RESm2). 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.

Stem volume over bark for year three, four, and five (VOLp3, VOLp4, and VOLp5) was calculated from the height and diameter measurements using the volume formula by Smalian cited by Riaño-Melo and Iván Lizarazo (2016):

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

where π = 3.1416; D_1 = large-end diameter (cm^2); D_2 =small-end diameter (cm^2); L^2 = length (cm^2).

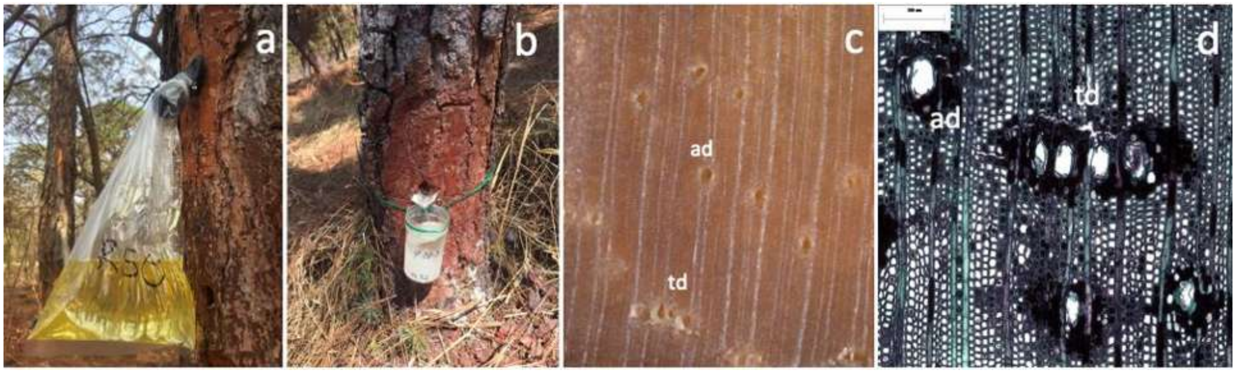


Fig. 3.2 Resin yield and anatomical assessment.

a, evaluation of mother trees of *P. oocarpa* **b**, evaluation of progenies of *P. oocarpa* by microchipping **c**, wood sample of an adult tree of *P. oocarpa* before processing in the laboratory (transverse plane with a Zeiss microscope (Axio Zoom V16) visible axial and traumatic resin ducts in various growth rings **d**, traumatic ducts in cross-section, axial parenchyma with tannins. Scale bar is 2 mm in **c**; 300 µm **d**

Selection and collection of samples for anatomical studies. The material for the anatomical analyses was recollected from 74 individuals in the progeny test 30 cm above the soil surface and 10 cm above the wound done during the microchipping. We chose a subset of four high yielding families and four low yielding families. The control group was formed with individuals from the protection barrier, the rows of trees for controlling the border effect, which were never tapped before. We also collected samples from 20 mother trees phenotypically selected for high resin yield and four control trees. Samples were extracted with a metal punch, stored in formalin-acetic acid-ethanol (FAA), and then prepared for microscopy observation using standard techniques (Ruzin 1999). Microphotographs were used to measure the resin duct traits. We measured the area and Feret diameter of the traumatic ducts (TD) and established a coefficient of total area (number of axial/radial ducts per area) in the phloem and xylem tissues (Areatot).

Statistical analysis. To estimate differences among the means of the families ($p \leq 0.05$), we performed analyses of variance (ANOVA). The analyses of the variance were performed by MIXED 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:

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

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 average value of the experiment; βi is the effect of the i-th block; φj is the random effect of the j^{th} family [$E(\varphi_j) = 0$; $Var(\varphi_j) = \sigma^2_f$]; βφij 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 [$E(\varepsilon_{ijk}) = 0$; $Var(\varepsilon_{ijk}) = \sigma^2_e$].

For the analysis of the subset of the anatomical traits (traumatic ducts and total area), the following expression was used:

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

The descriptive statistics of each one of the studied anatomical characteristics were obtained. However, due to the fragility of the tissue, 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 1984):

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

$$h_f^2 = \sigma_f^2 / \left[\sigma_f^2 + (\sigma_{bf}^2/b) + (\sigma_e^2/nb)\right]$$

$$h_w^2 = \frac{3\sigma_f^2}{\sigma_e^2}$$

where $\sigma^2{}_f$ = family variance (additive), $\sigma^2{}_{bf}$ = block variance per family, $\sigma^2{}_e$ = residual variance, b = number of blocks, *n* = 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:

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

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

where *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.

Genetic and phenotypic correlations between all the analyzed characteristics of the same age and at different ages (among 3, 4, 5, and 6 years) were analyzed. The phenotypic correlations were estimated by the Pearson correlations coefficients using the CORR procedure. The genetic correlations $r_{g(x,y)}$ between the pair of variables we used the formula indicated by Falconer and Mackay (1996):

$$r_{gxy} = \frac{\sigma_{fxy}}{\sigma_{fx} \ \ \sigma_{fy}}$$

Also, the standard error of the genetic correlations was estimated by the equation proposed by Falconer and Mackay (1996):

$$SE(r_g) = 1 - r_g^2 \frac{\sqrt{SE(h_{ix}^2)SE(h_{iy}^2)}}{2h_{ix}^2 \ h_{iy}^2}$$

where $r_{gxy}{}^2$ is the genetic correlation between the trait x and y, $SE(h^2{}_x)$, and $SE(h^2{}_y)$ are standard errors of the narrow-sense heritability of the trait x and y, respectively, and $h^2{}_x$ y $h^2{}_y$ are the heritability coefficients of the variables x and y.

The genetic gain was estimated by the following formula (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 morphological, anatomical, and resin yield to consider the possible associations. Linear regressions were measured to assess the relationships between variables to explore if resin yield can be predicted based on any of the analyzed characteristics. The traits considered in the estimation of the possible relations between variables are detailed in **Table 3.1**.

Table 3.1 Descriptions of traits considered for the correlation analyses in this study and their abbreviations.

Trait	Description	Abbreviation	Unit
Morphological traits	Total height - progenies (age in years: 3, 4, 5, i)	HTp 3, 4, 5, i	m
	Basal diameter - progenies (age: 3, 4, 5, i)	BDp 3, 4, 5, i	cm
	Breast height diameter - progenies (age: 3, 4, 5, i)	DBHp 3, 4, 5, i	cm
	Stem volume - progenies (age: 3, 4, 5, i)*	VOLp 3, 4, 5, i	m^3
	Breast height diameter mother trees	DBHm	cm
Anatomical traits*	Area axial ducts (p)	AD area	mm^2
	Area radial ducts of xylem (p)	RDX area	mm^2
	Area radial ducts of phloem (p)	RDP area	mm^2
	Density of axial ducts/mm^2 (p)	AD density	mm^2
	Density radial ducts of xylem/mm^2 (p)	RDX density	mm^2
	Density radial ducts of phloem/mm^2 (p)	RDP density	mm^2
	Area traumatic ducts (p) *	TD area	mm^2
	Feret´s diameter traumatic ducts (p) *	TD diam	mm
	Axial duct coefficient (m + p) *	AD tot area	mm^2
	Xylem radial duct coefficient (m + p) *	RCX tot area	mm^2
	Phloem radial duct coefficient (m + p) *	RCP tot area	mm^2
Resin yield	Resin mother trees evaluation 2011 (5 months)	RESm1	kg/tree
	Resin mother trees evaluation 2017 (48 ^{hours}) *	RESm2	g/tree
	Resin progenies year yield (age 3, 4, 5, 6, and mean across the four ages) *	RESp 3, 4, 5, 6, i	g/tree

(i = mean across the four ages (3,4,5,6); m=mother trees; p=progenies; * we marked with an asterisk the traits used to estimate the genetic parameters)

3.3 Results

Trends in annual means traits. The present study assessed the genetic variations in resin yield, growth, and anatomical traits as well as the phenotypic and genetic correlations between these traits among 27 half-sib families at different ages (age year three, four, five, and six), and the possible genetic gain. We explored the variation of resin yield among half-sib families and individuals. The mean values, standard deviation of the mean, the coefficients of variation, and range of variation for all traits over the assessed years are presented in **Table 3.2**.

Table 3.2 Comparison of mean values of half-sib families for resin yield and volume, traumatic ducts and resin duct coefficients of *P. oocarpa* at various years and across years in Mich., Mexico.

Variable	N	Mean	St. dv.	Min	Max	CV	Fam amp	Fam amp %
RESm2	75	95.42	99.06	8.24	654	103.81	285.22	95
RESp3	369	3.58	3.74	0.01	23.43	104.54	10.68	91
RESp4	423	4.08	4.21	0.01	22.99	103.12	7.37	84
RESp5	371	8.36	7.39	0.01	41.8	88.39	14.1	76
RESp6	361	12.97	11.82	0.02	60.9	91.17	24.23	76
RESp(i)	436	6.8	4.7	0.01	30.28	69.15	11.64	82
VOLp3	383	0.005	0.003	0.0003	0.020	58.46	0.004	45
VOLp4	418	0.02	0.01	0.0006	0.07	51.23	0.02	48
VOLp5	374	0.07	0.03	0.0037	0.16	46.75	0.05	49
VOLp (i)	421	0.02	0.01	0.0003	0.07	52.14	0.02	48
TDp area	40	0.015	0.003	0.007	0.025	23.45	0.007	65
TDp diameter	40	0.182	0.024	0.106	0.236	13.24	0.517	24
ADp tot area	69	0.015	0.005	0.006	0.029	31.04	0.0036	22
RCXp tot area	70	0.002	0.001	0	0.003	35.1	0.0005	26
RCPp tot area	62	0.002	0.001	0.001	0.003	37.74	0.0007	35
TDm area	34	0.026	0.031	0.006	0.161	120.482	0.007	65
TDm diameter	30	0.237	0.12	0.12	0.626	50.769	0.0517	24
ADm totarea	22	0.0123	0.0106	0.0032	0.0442	86.54	40,999	93
RDXm totarea	24	0.0012	0.0006	0.0005	0.0026	45.836	1,372,698.05	21
RDPm totarea	21	0.0011	0.0005	0.0005	0.0024	47.222	1,887,220	20

For variable abbreviations, see **Table 3.1**, mean = mean values, St. dv. = standard deviation, Min = minimum values, Max = maximum values, CV = coefficient of variation (%), amplitude of families (the largest mean value among the families minus the lowest mean value), RESm (g), RESp (g), volume (VOL, m^3), traumatic ducts (TD, area=mm^2; diameter=mm), coefficient (average area*average number of ducts=mm^2); Fam amp = family amplitude (differences between the mean value of the best performing family minus the mean value of the worst performing family); Fam amp % = family amplitude as percentage, m=mother trees; p=progenies; i=interannual mean across years.

With the traditional tapping method, an average yield of 2.19 kg/month/tree was obtained (Reyes-Ramos et al. 2019). With the experimental method for resin extraction on the mother trees, we got an average yield of 318 g/tree in 48 hours. The sample was not contaminated, and losses were minimal. We harvested a maximum of 906 grams in total (family 16) and a minimum of 46 grams (family 8). The average resin yield values per year per family are presented in **Fig. 3.3**. The phenotypic correlation between both evaluations (conducted in years 2011 and 2017, respectively) was moderate and positive ($r_p = 0.60$) and a linear regression was obtained with a $R^2 = 0.36$ ($p > 0.05$).

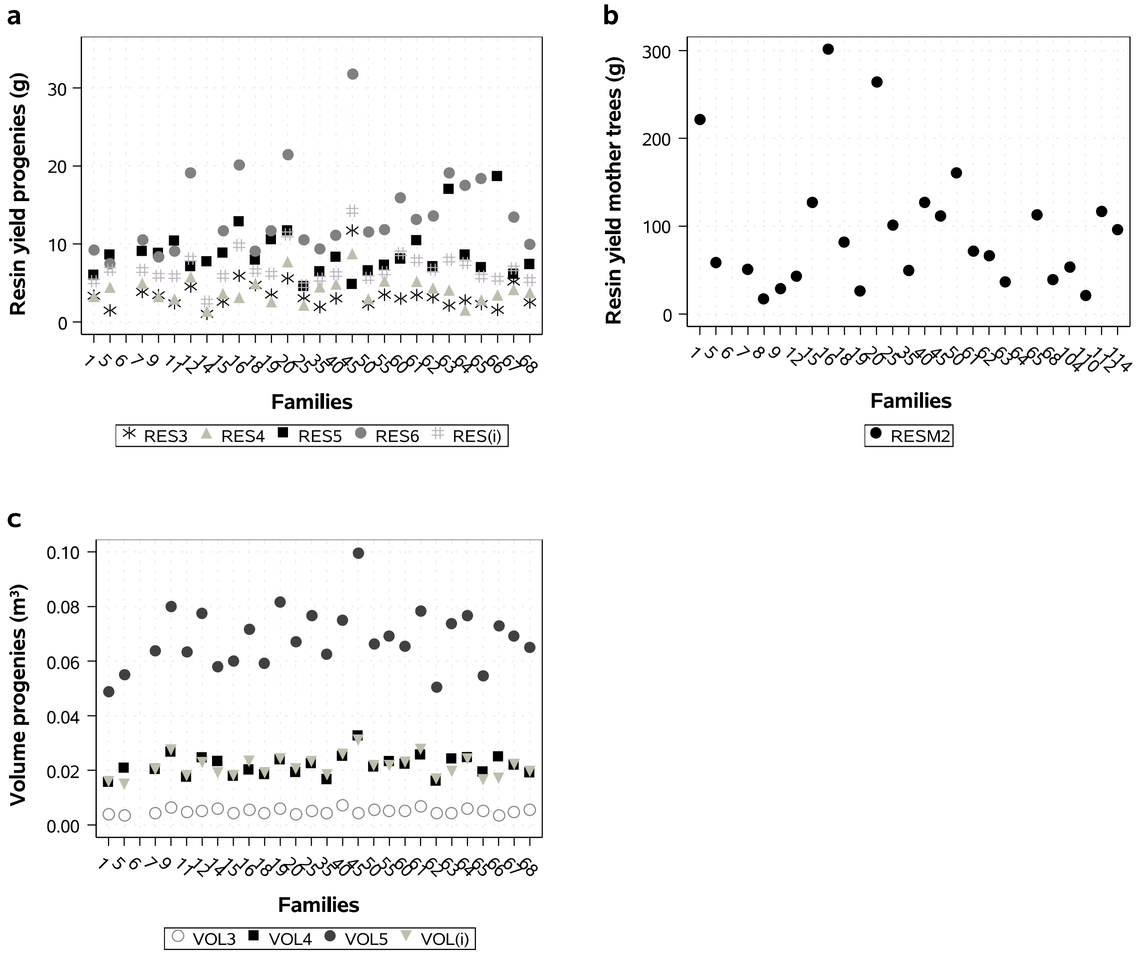


Fig. 3.3 Mean values per year per family for resin yield and stem volume in a progeny trial of *P. oocarpa* in Michoacán, Mexico.

a, mean resin yield of the progenies, **b**, mean resin yield of the mother trees (48 hours assessment), **c**, mean stem volume of the progenies

The DBHm mean annual growth rate for the mother trees between year 2011 and 2017 was 1.85 cm per year. Additional information about the first evaluation is detailed in Reyes-Ramos et al. (2019). Here we only present data about the second evaluation. We measured 24 trees, the average diameter at breast height (DBHm) was 62 cm.

Among the families in the progeny trial, mean resin yield increased with age, from year three to year six by 262%. The average yield of the progenies ranged from 3.6 to 13.0 g per year and 6.9 g (*SE* ± 0.43) as average across the years. In the progenies, the mean values for volume increased gradually from year three to year five

(VOLp 3 to 5 = 0.005 to 0.07 m^3). The fastest-growing family in the progeny trial across years reached a mean volume of 0.03 m^3 (family 45) and the slowest growing a mean volume of 0.01 m^3 (family 5).

The traumatic ducts were easily identified by the naked eye and were arranged in tangential bands of three ducts up to seven ducts per band. The mean number of tangential bands per sample was 2.44. The average area of the traumatic ducts in the progenies was 0.015 mm^2 and 0.026 mm^2 in the mother trees. Feret´s diameter had a length of 0.182 mm in the progenies, and 0.237 mm in the mother trees. Ducts were present in wounded and non-wounded trees. In the mother trees, traumatic ducts were found only in 9 samples, while they were present in 50 samples of the progenies. However, only six samples of the mother trees and 39 samples of the progenies were fully processed to measure the ducts, due to the greater fragility of the tissue (**Table 3.2**). The mean total area of the coefficient of the axial ducts ranged from 0.015 in the progenies to 0.012 mm^2 in the mother trees. For the radial ducts, the coefficient (xylem and phloem) ranged from 0.002 mm^2 to 0.001 mm^2 (**Table 3.2**).

We found statistically significant differences among families for resin yield in all the assessed years, with a probability level of 5%, except for year five. Among blocks (except for year three) and for block x family interaction, no statistically significant differences were found. We also observed significant differences among families for resin yield for the mother trees in the second evaluation (48 hours). Stem volume showed significant differences for years four and five. As opposed to resin yield, there were statistically significant differences for block on stem volume, but there was no significance for the block-family interaction (**Table 3.3**)**.** As for the assessed subset of anatomical traits, there were statistically significant differences among families, for the area and diameter of the traumatic ducts, but not for any other resin duct trait (coefficients) (**Table 3.3**)**.**

Table 3.3 Genetic parameters of a progeny trial of *P. oocarpa* in Mich., Mexico.

Significance of the ANOVA (*p*), variance components $\sigma^2_{f\%}$ = component of family variance; $\sigma^2_{b\%}$ = component of block variance; $\sigma^2_{bf\%}$ = component of block*family variance; $\sigma^2_{e\%}$ = component of error, *SE* = standard errors for heritabilities (at individual and family mean level), $CV_{g\%}$ = coefficients of genetic variation, $\Delta G/y_{\%}$ = genetic gain for resin yield and stem volume.

Age	N	Variance components %										
		$\sigma^2_{f\%}$	p	$\sigma^2_{b\%}$	p	$\sigma^2_{bf\%}$	p	$\sigma^2_{e\%}$	h^2_i	h^2_f	h^2_w	$\Delta G/y_{\%}$
RESm2 (g)	24	34.1	.	.	.	.	.	66	.	.	.	.
RESp3 (g)	369	4.4	0.004	5.6	0.001	3.5	0.010	86.5	0.14(0.03)	0.44(0.13)	0.15	20.7
RESp4 (g)	423	6.1	0.006	1.6	0.232	0.0	0.491	92.3	0.19(0.04)	0.54(0.15)	0.20	27.0
RESp5 (g)	371	3.8	0.065	3.0	0.106	0.0	0.903	93.2	0.12(0.03)	0.39(0.12)	0.12	14.9
RESp6 (g)	361	6.7	0.000	0.0	0.350	0.0	0.135	93.3	0.20(0.04)	0.52(0.22)	0.22	25.2
RESp (i) (g)	436	7.3	0.000	4.7	0.008	1.7	0.205	86.4	0.23(0.04)	0.59(0.25)	0.25	22.3
VOLp3 (m^3)	383	4.5	0.053	24.7	<.0001	24.7	0.060	46.2	0.25(0.04)	0.46(0.16)	0.29	12.1
VOLp4 (m^3)	418	2.2	0.048	12.0	<.0001	8.5	0.211	77.3	0.08(0.03)	0.29(0.10)	0.09	5.7
VOLp5 (m^3)	374	3.1	0.041	12.6	<.0001	8.8	0.227	75.4	0.12(0.03)	0.35(0.11)	0.12	7.7
VOLp(i) (m^3)	421	2.6	0.035	11.5	<.0001	10.5	0.202	75.4	0.10(0.03)	0.32(0.11)	0.10	7.1
AD Areatot (mm^2)	40	0.0	0.880	0.0	0.789	.	.	100.0	.	.	.	.
RDX Areatot (mm^2)	40	0.0	0.509	0.0	0.890	.	.	100.0	.	.	.	.
RDP Areatot (mm^2)	69	5.6	0.335	0.0	0.186	.	.	94.4	.	.	.	.
Area traum (mm^2)	70	30.1	0.013	1.9	0.167	.	.	68.1	.	.	.	.
Diam traum (mm)	62	49.6	0.001	0.0	0.413	.	.	50.4	.	.	.	.

Genetic variability and heritability. The estimates of components of variances, coefficients of genotypic, residual, and phenotypic variations, and genetic gains for resin yield and stem volume at all ages are presented in **Table 3.3**. The contributions of the components of family variance to the total variance were higher for resin and lower for stem volume (except for year three, where there were no significant variances among families) but were highest for some of the anatomical traits (traumatic ducts). The family effect contributed to total variance 4 to 7% for resin yield, and 2 to 4% for stem volume. The contribution of the family effect to the total variance for resin duct traits ranged from 5.6 to 49.6% and the variance error from 50.4 to 100%. (**Table 3.3**).

Narrow sense heritability (h^2_i) estimates for resin yield ranged from 0.14 to 0.20 and was much lower than family means heritability coefficients. The heritability at family mean level (h^2_f) varied from medium to high (0.39 to 0.54), which suggests that 44% to 54% percent of the variation observed in the family means is attributable to genetic differences and that genetic gain for resin yield can be expected through a family selection in *P. oocarpa* (Liu et al. 2013). The highest values of heritability at family mean level was found in the fourth year of the progeny test. The heritability for resin yield across years was higher than for stem volume. However, they presented a slight fluctuation and peaked at year four as opposed to stem volume where the coefficients were highest at year three and then decreased, although in year three there was no significant variation among families. Narrow sense heritability (h^2_i) estimates for stem volume varied from 0.08 to 0.25, and family mean heritability (h^2_f) ranged from 0.29 to 0.46 (**Table 3.3**). The standard errors were relatively low for individual-tree heritability (±0.03), unlike the ones estimated for family-mean heritability (±0.12), which were slightly higher. Since heritability coefficients of the growth traits: basal diameter, diameter at breast height and total height were discussed in another article (Fabián-Plesníková et al. 2020), here we present only detailed genetic parameters for stem volume.

Phenotypic, genotypic, age-age correlations, and genetic gain. Genetic correlations are shown in (**Table 3.4**). The highest positive correlation was found for the association of DN-RY (0.56). Resin yield was more strongly genetic correlated with

DBH (r_g = 0.56) than with tree height across ages (r_g = 0.22). The phenotypic and genetic correlations between DBH and resin yield were all significant ($P < 0.05$). We found low and positive phenotypic correlations with morphological traits (height, basal diameter, breast height diameter) (r_P= 0.12 to 0.36) (**Table 3.5**). The highest phenotypic correlations were found in the third year and in the subsequent years they decreased. The age-age correlations among resin yield were high and positive, the values decreased as the difference in age increased, as it is generally expected (**Table 3.6**). Genetic gain was the highest for resin yield $\Delta G/y_{\%}$ = 15% to 35% at a selection intensity of the best trees of i = 1.4, and potential gains are presented in **Table 3.3**.

Table 3.4 Genetic correlations.

r_g	***ALT-RESp***	***DIAM-RESp***	***DN-RESp***	***VOL-RESp***
3	-0.76±0.06	-0.41±0.32	-0.97±0.38	.
4	0.01±0.13	-0.76±0.25	-0.22±0.17	-0.85±0.32
5	0.21±0.12	0.20±0.11	0.56±0.07	0.21±0.14
Mean (i)	0.22	0.27	0.65	0.28

Variable abbreviations as coded in Table 1.

Table 3.5 Phenotypic correlations.

r_P	***ALT-RESp***	***DIAM-RESp***	***DN-RESp***	***VOL-RESp***
3	0.23	0.24	0.36	0.25
4	0.12	0.12	0.17	0.19
5	ns	ns	0.11	0.11
Mean (i)	0.23	0.22	0.31	0.27

Variable abbreviations as coded in Table 1.

Table 3.6 Age-age correlations of resin yield of progenies.

Traits	Resin yield			
	RESp14	**RESp15**	**RESp16**	**RESp17**
RESp14	.	0.55	0.92	1.27
RESp15	.	.	0.60	0.84
RESp16	.	.	.	0.99
Resp17	.	.	.	.

Variable abbreviations as coded in Table 1.

Family assessment. The family with the highest yield (across years) overcame the progeny with the lowest yield on average by 82%. This shows a large amount of the differences among the families of the progenies. On the other hand, for stem volume, the difference between the fastest-growing progeny and the slowest was lower (48%).

The correlation of the assessment of the resin yield between years was significant but moderate. **Table 3.7** shows the relative position of the progenies at age three, four, five, and six for resin yield and three, four, five for volume with their respective hierarchical position.

Table 3.7 Relative position of the progenies of mayor volume and resin yield in a progeny test of *P. oocarpa* by year.

Resin yield					Volume			
3	**4**	**5**	**6**	**Average across years**	**3**	**4**	**5**	**Average across years**
45	45	66	45	45	40	45	45	45
16	60	63	20	20	61	9	19	61
20	20	16	16	16	9	61	9	9
67	12	20	63	60	19	40	61	40
18	55	19	12	12	14	66	12	19
12	61	61	65	63	64	64	25	64
7	7	11	64	61	68	12	64	16
19	18	7	60	64	16	63	40	25
55	40	15	62	67	50	19	63	60
61	35	9	67	62	12	14	66	12

3, 4, 5, 6 = indicates the age of trial

The mean resin yield of the highest yielding family in the present study exceeded 4.5 times the average of the lowest yielding family and doubled the general average yield. For stem volume, the fastest growing family doubled the volume of the slowest growing family. The general average of the volume was surpassed 1.5 times by the fastest growing families.

Association of resin yield with morphology and anatomy

Resin yield *vs.* morphology. The coefficients of regressions between resin yield and growth traits ranged from 0.22 to 0.34. Not every year, the associations were statistically significant, so we are presenting just significant relationships (**Table 3.8**).

Table 3.8 Linear regressions of morphological traits and resin yield.

	RES_3	RES_4	RES_5	RES(i)
VOLp4	ns	ns	0.28	0.28
VOLp5	0.36	ns	0.36	0.29
VOL(i)	0.43	ns	0.30	0.29
DBHp3	0.25	ns	0.23	ns
DBHp4	0.36	0.35	0.35	ns
DBHp5	0.32	ns	0.32	0.35
DBHp(i)	0.20	ns	ns	ns
DIAMp4	ns	ns	0.22	ns
ALTp5	0.32	ns	0.28	0.29
ALTp(i)	0.34	ns	0.22	0.26

Variable abbreviations as coded in Table 1.

Resin yield *vs*. anatomy. In general, resin yield was related only to three constitutive resin duct features: area and Feret´s diameter of axial ducts in the xylem (AD) and density of the xylem axial ducts. However, there was also a high and positive correlation for the association of the area and Feret´s diameter of the traumatic ducts with resin yield of the year five (RESp5 r_p^2 = 0.76 to 0.88) (**Fig. 3.4**). Resin yield of the mother trees (RESm2) was also highly and positively correlated to several anatomical characteristics such as area and diameter of traumatic ducts, as well as to the area and normal diameter of axial ducts (r_p^2 = 0.75 to 0.86). Resin flow of the mother trees in both years of assessment (RESm1 and RESm2) was positively correlated to the area of xylem axial ducts and Feret´s diameter of the traumatic ducts of the progenies. Linear regressions were run to understand the effect of the anatomical features on resin yield, and we found differences between high yielding families and low yielding families for anatomical variables (**Fig. 3.5**).

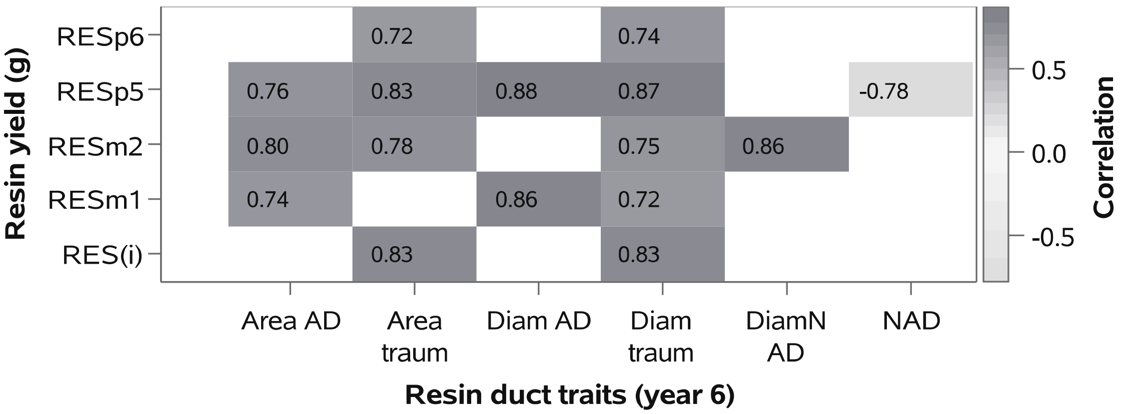
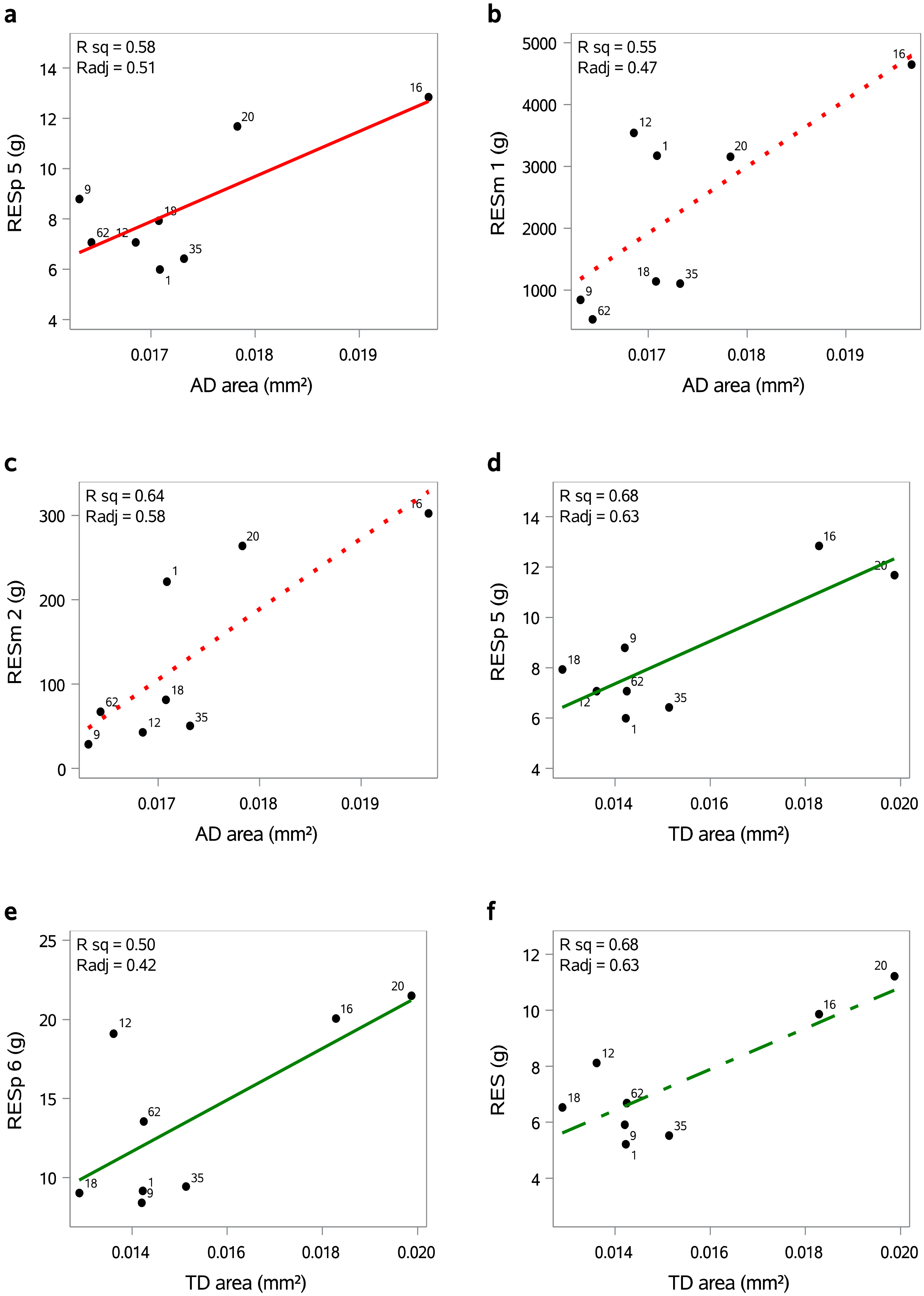


Fig. 3.4 Pearson´s correlation between anatomical traits and resin yield.

The intensity of the gray color indicates the intensity of the correlation. Area AD = area of axial ducts (mm^2); Area traum= area of traumatic ducts (mm^2); Diam AD = Feret´s diameter of axial ducts (mm); Diam traum = Feret´s diameter of traumatic ducts; DiamN AD = normal diameter of axial ducts (lumen and epithelial cells measured by the traditional method), NAD = number of axial ducts per mm^2.


a
R sq = 0.58
Radj = 0.51
RESp 5 (g)
AD area (mm²)
4
6
8
10
12
14
0.017
0.018
0.019
16
20
9
18
62
12
35
1
b
R sq = 0.55
Radj = 0.47
RESm 1 (g)
AD area (mm²)
1000
2000
3000
4000
5000
0.017
0.018
0.019
16
12
1
20
18
35
9
62
c
R sq = 0.64
Radj = 0.58
RESm 2 (g)
AD area (mm²)
0
100
200
300
0.017
0.018
0.019
16
20
1
18
62
12
35
9
d
R sq = 0.68
Radj = 0.63
RESp 5 (g)
TD area (mm²)
4
6
8
10
12
14
0.014
0.016
0.018
0.020
16
20
9
18
62
12
35
1
e
R sq = 0.50
Radj = 0.42
RESp 6 (g)
TD area (mm²)
10
15
20
25
0.014
0.016
0.018
0.020
20
16
12
62
18
1
9
35
f
R sq = 0.68
Radj = 0.63
RES (g)
TD area (mm²)
4
6
8
10
12
0.014
0.016
0.018
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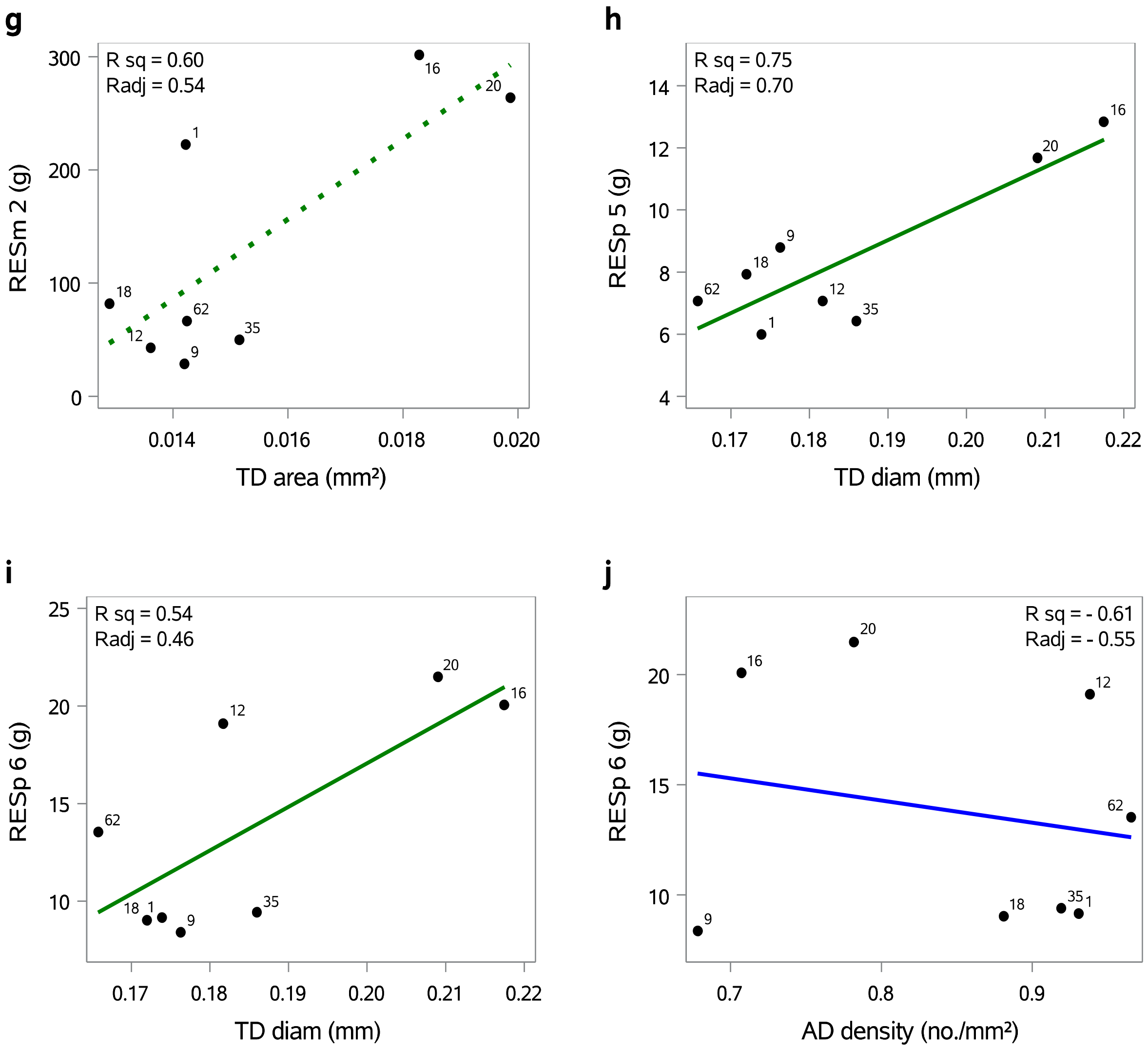

Fig. 3.5 Linear regressions between resin yield and morphological and anatomical traits.

a) axial duct area and resin yield of the progenies year five, **b)** axial duct area and mother trees 1st evaluation (5 months); **c)** axial duct area and mother trees 2nd evaluation (48 hours); **d)** traumatic duct area and resin yield of the progenies year five **e)** traumatic duct area and resin yield of the progenies year six; **f)** traumatic duct area and resin yield across the year (interannual mean); **g)** traumatic duct area and resin yield mother trees 2nd evaluation (48 hours); **h)** traumatic duct diameter and resin yield of the progenies year 5**; i)** traumatic duct diameter and resin yield of the progenies year six; **j)** axial duct density and resin yield of the progenies year six.

3.4 Discussion

Evaluation of the productive potential of the selected trees and their variation. The trial presented good production performance mainly for resin yield and enough genetic variability among half-sib families to proceed with a selection of the best progenies and/or the best mother trees, in order to develop a sexual or asexual seed orchard, respectively. To achieve genetic gains in resin productivity for *P. oocarpa* in the region is viable since there was a significant genetic variability for resin yield among mother trees ($p = 0.002$) and among their progenies ($p = 0.0001$), which is an essential factor in providing the best seed sources (Dorman 1974). Although the present results are based on a single progeny test established at one site, the genetic parameter estimates are based on tested families over four years.

The high variability among parent trees for resin yield and the fact that we found in the progeny trial differences among families for both traits (resin yield and stem volume) agrees with what was found in previous studies on *P. oocarpa* (Wright and Osorio 1992; Moura et al. 1998; Sampaio et al. 2002), and other species such as *P. massoniana, P. caribaea var. hondurensis,* and *P. elliottii* (Liu et al. 2013; Zeng et al. 2013; Santos et al. 2016b; Lai et al. 2017). Similarly, with other reports, resin yield presented a higher degree of genetic variation among half-sib progenies, as opposed to stem volume, which showed a lower degree of variation (Lai et al. 2017). As for the anatomical characteristics, the only traits that showed important variation among progenies were the area and the diameter of the traumatic ducts.

The low amount of variation for volume (in comparison with resin yield) could be a consequence of the fact that parent trees were selected for high resin yielding ability only (Squillace and Bengtson 1961).

Our data on the second assessment of the mother trees (RESm2) agree with what was reported for *P. elliottii*, where they found that it is possible to evaluate trees to identify and select high yielding trees just for a short period of time (several hours; 48 hours in our case) and use the results for the establishment of more productive commercial plantations and/or reforestations (Neis et al. 2019). This method of assessment could possibly shorten periods of evaluation in the first phase of the breeding programs and significantly lower the costs during the selection of plus trees for resin yield.

Studies on growth traits on *P. oocarpa* have been done all over the world, particularly the most important program initiated in the 70´s under the Oxford Forestry Institute. However, only very few of them focused on the resin yield of the species. The estimated maximum value at the family level in the last year of evaluation in the present study for resin yield (max = 31.7 g, 6 years) was similar to those observed by Liu 2005 (max = 30.6 g, 11 years) in *P. massoniana*, but much higher than those detected by Liu et al. 2013 (max = 11.8 g, 8 years, 45 families), and Zeng et al. 1998 (max = 8.9 g, 7 to 13 years, 48 families) in the same species. Nevertheless, mean resin yield (mean= 13.8 g, 6 years) was lower than in Liu 2005 (mean= 18.3 g, 8 years), but higher than in the studies by Zeng et al. 1998 and Liu et al. 2013 (mean= 7.2 g; 8.7, respectively). The difference in resin yield between this study and the previously mentioned trials may be due to differences among the species studied, the families being compared and the number of families in the trials, as well as site effects.

The residual error was the most important source of variation for all traits, indicating a significant degree of variation among trees within half-sib families, which makes possible a selection within them (Hernández-Máximo et al. 2016). The variance fluctuated across ages, but not abruptly, so that the differences were relatively stable as age increases. The family variance, which corresponds to 1/3 of additive variance (Wright 2012), was the highest for resin yield and significantly lower for stem volume. The family effect was the second most important source of variation for resin yield (except for year three), whereas for stem volume, the family effect was the lowest source of variation, when compared with the other sources of variation (block, etc.).

Family effect was the highest for the traumatic ducts, although it must be considered that this effect could be overestimated because it was estimated from a subset of families selected either for high or low resin production.

Genetic control of resin yield, morphological and anatomical traits. The results of the estimates of genetic parameters prove that potential gains can be obtained with the improvement of *P. oocarpa* species for resin yield and wood. During the assessment period, narrow-sense heritability estimates on individual tree basis and within family were moderate for resin yield and low for volume (except for year three) according to scale determined by (Resende 2002) (where <0.15 is low, 0.15 to 0.50 is moderated and <0.50 is high). The estimated coefficients for resin yield were similar to the what was found in *P. massoniana* (Zeng et al. 2013; h^2_i = 0.13 to 0.20, 7 to15 year), but much lower than in several other studies: *P. elliottii* (Squillace and Bengtson 1961; h^2_i = 0.45 to 0.90, 10 to 11 years), *P. elliottii* (Pswarayi et al. 1996; h^2_i = 0.37, 15 year), *P. merkusii* (Susilowati et al. 2013; h^2_i = 0.58, 30 year), *P. massoniana* (Liu et al. 2013; h^2_i = 0.38, 8 year), and *P. caribaea* (dos-Santos et al. 2016; h^2_i = 0.39, 26 year). But it was similar with what was found for *P. elliottii* across three sites (Lai et al. 2017; h^2_i = 0.11 (0.10, 0.21, 0.41 – for each site separately), 27 year).

Stem volume heritability values were much lower to other studies (Resende 1995; Matziris and Zobel 1973; Pswarayi et al. 1996; Hodge and Dvorak 1999; Sampaio et al. 2002)**.** The relatively low heritabilities observed in the present study for these traits suggest that either volume is under strong environmental influence (likely) or that the numbers of progeny tested trees should be higher (Squillace and Bengtson 1961).

Heritability estimates based on family means were moderate to high for resin yield (h^2_f =0.59), and moderate for volume (h^2_f = 0.32) across ages, being generally higher than estimates at individual and within family level. The coefficients estimated at family mean level for both traits (resin and stem volume) were higher than individual narrow sense heritability, revealing that a selection of these characters could be more effective at the level of families than by individual tree and within families (Sampaio et al. 2002). This was also found by other authors in other pine species such as *P. elliottii* (Lai et al. 2017). Vencovsky and Barriga (1992) consider experimental errors are much lower when means are used in the selection criteria; thus, estimates using the means have higher accuracy when selecting genotypically superior progenies for the variables of interest (Vencovsky and Barriga 1992). The lower magnitude of the coefficients of heritability in stem volume indicates that the environmental effects still have a predominant influence over this characteristic, at least in comparison with resin yield. Also, it possibly reflects the fact that the selection was done based on resin yield only.

The estimates of the coefficients of heritability at family mean level for resin yield were similar to values reported for other species *P. massoniana* (Zeng et al. 2013; Liu et al. 2013; h^2_f = 0.52 to 0.59, 7 to 15 year; h^2_f = 0.47, 8 year), *P. caribaea* (dos-Santos et al. 2016; h^2_f = 0.62, 26 year), and on *P. elliottii* (Lai et al. 2017; h^2_f = 0.41, across sites). However, they were lower than for *P. merkusii* (Susilowati et al. 2013; h^2_f = 0.70, 30 year). Volume values were much lower than for *P. massoniana* (Zeng et al. 2013; h^2_f = 0.43 to 0.55, 7 to 15 year), *P. caribaea* in Brazil (dos-Santos et al. 2016; h^2_f = 0.62, 26 year), and *P. elliottii* in China (Lai et al. 2017; h^2_f = 0.42, 27 year).

Higher values for resin yield suggest that genetic factors control predominantly this character (Susilowati et al. 2013). This pattern also indicates that early breeding programs aimed to increase resin yield in *P. oocarpa* can begin by a phenotypic selection (indirect selection) of individual high-yielding trees (Susilowati et al. 2013), and that there is a potential for genetic progress, since it has been confirmed by previous studies that it is a highly heritable trait (Gurgel-Garrido et al. 1996; Tadesse et al. 2001; White et al. 2007).

Relationship of resin yield with other growth quantitative traits. The genetic correlations between the growth characteristics and resin yield in the present study were low or high and negative except for the association between diameter at breast height (DBH) and resin yield across ages (r_g = 0.65). The moderate and positive association between DBH and resin yield shows that genetic factors possibly act simultaneously to increase resin production and DBH (dos-Santos 2016), so the selection of one trait increases the other (Santos et al. 2014).

In general, genetic correlations were higher than the corresponding phenotypic correlations, which indicates that if we use phenotypic correlations to evaluate efficiency of this early selection this could lead us to a possible underestimation of the potential gains from this selection (Vargas-Hernandez and Adams 1992; dos-Santos et al. 2016).

Previous studies show mixed results on the genetic correlation between resin yield and growth traits. Low correlations for height and basal diameter agree with what was observed in studies on *P. elliottii* var. elliottii in Brazil (Gurgel-Garrido and

Kageyama 1993; Romanelli 1995), *P. caribaea* (Santos et al. 2016a), but are opposite to what was found for *P. elliottii* in China, where growth traits (diameter at breast height and height) presented a strong and positive correlation with resin yield (Pswarayi et al. 1996; Tadesse et al. 2001). Overall, the resource availability hypothesis (RAH) and growth-differentiation balance hypothesis (GDBH) are the main explanations for the relationship between RY and growth in pines (Lai et al. 2017). The negative genetic correlations could evidence that in the first years the trees have to invest in accumulating biomass, rather than in their defensive system.

Can the selection cycles of superior genotypes in resin production be shortened? Estimates of age-age correlations for resin yield in previous studies revealed also high and significant values from 0.55 up to 0.90, suggesting that the repeatability of the trait is high and there is a potential for early selection, and there is no need to wait for older ages for the final selection stages (Squillace and Gansel 1974; Gurgel-Garrido and Kageyama 1993; Gurgel-Garrido et al. 1994; Romanelli 1995; Romanelli and Sebben 2004; Zeng et al. 2013). The correlations in the present study also point in that direction suggesting that trees that showed the highest resin yield, in early stages, could repeat the performance in more advanced ages (Pswarayi et al. 1996; Romanelli and Sebben 2004).

Productive potential of the selected trees. From the studied families, 8 families showed higher resin yield than the average, and 10 families surpassed the mean values in volume, which indicates there is a large variance among families. These data suggest there is a large potential for selection for resin yield productivity and confirms that the selected progenies are a good base for breeding. In this study variation among the studied families of *P. oocarpa* was larger for resin yield than for other species such as *P. elliottii*, were resin yield of the best family doubled the resin yield of the worst family (out of 113 families) (Zhuang et al. 2007).

Three of the highest yielding families showed some stability in their performance across years in resin production and two for volume. Family coded 20 being ranked 3rd, 3rd, 4th, and 2nd respectively in each year of assessment by microchipping, and 2nd when averaging across years. The parental mother-tree of this family was also the second highest yielding tree in the 48 hours assessment. As for progeny family coded 16, mean resin values were ranked 2nd, 16th, 3rd, 3rd and 3rd across years. As for the parental mother-tree coded 16, it was the highest yielding tree in both the first 5 month and the second 48 hours assessment. And family 45 ranked 1st, 1st, 26th, 1st, and 1st across years. However, the parent tree ranked 2nd in the 5-month assessment and 9th in the 48^{-hour} assessment.

For stem volume the performance of two families was relatively stable over the years. Family coded 45, which also ranked in the top three families for resin yield, ranked in the first year in the 18th place, then it moved up to the 1st place for the two consecutive years, and it was also in the first position on the average across years. Another family was the Family coded 9 which ranked 3rd, 2nd, 3rd, and 3rd across years.

The observed results indicate the highest gain in the next generation of breeding can be obtained for resin yield, particularly, at year four (27%) and for stem volume at year five (7.7%), since there was no variation among families at year three (12%). The high values agree with what was found in most of the progeny tests for *P. elliottii* pine resin yield (Wright 2012).

Anatomical and morphological traits associated with resin synthesis. In general, it has been noted that the resin duct system of the trees (number and size of resin ducts in the xylem and phloem) influences resin biosynthesis, as it’s the site of production, storage, and mobilization of resin to the injured or attacked site (Hood and Sala 2015). The importance of these anatomical traits has been observed in several studies (Kane and Kolb 2010; Rodríguez-García et al. 2014; Ferrenberg et al. 2014; Gaylord et al. 2015; Hood and Sala 2015, Mason et al. 2019, Neis et al. 2019).

We observed in the present study that higher-yielding families presented a high and positive correlation between axial duct area with resin yield, but negative one with density (its number per mm^2). A higher amount of resin synthesis suggests that the tree has a greater probability of overcoming its natural enemies, since resin is an essential chemical defense against mechanical wounds, herbivores, and pathogens (Hodges et al. 1979). This is in alignment with what was reported by Zhao and Erbilgin (2019) on

P. contorta. They observed that trees with larger but fewer constitutive resin ducts had a greater probability of survival during an outbreak of mountain pine beetle attacks. Similarly, in a study on *P. loblolly,* they found a negative correlation between resin flow (constitutive and induced) and the number of resin ducts per cm (Lombardero et al. 2000). The high and positive correlations of the area of the ducts (xylem axial and traumatic) could be explained by the Hagen–Poiseuille equation, which implies that even a small increase in diameter has an important impact on conducting capacity of ducts (Schopmeyer et al. 1954; Sperry et al. 2006). On the other hand, the negative correlation of the density (number of ducts per mm^2) could suggest that there is a possible trade-off between size and number of resin ducts in pine trees (Herms and Mattson 1992) cited by Zhao and Erbilgin (2019).

Noteworthy, we observed that the area of the xylem axial ducts was associated with resin yield from the previous year for which the wood cores were sampled. However, we did not find a correlation between resin duct traits with resin yield of the same year the wood cores were sampled. The reason could be that climatic conditions of the previous year influence the traits of the resin duct system in the following year (thus the amount of resin yield) as it was observed in a study on *P. pinaster* (Rodríguez-García et al. 2014).

It is also interesting to note, that of all the analyzed traits, the best correlation was found between both types of resin ducts, the constitutive (i.e. axial canal area) and the induced (i.e. traumatic duct area/diameter) with resin yield capacity. The high and positive association observed can possibly be explained by the fact that trees with high levels of constitutive defenses could tend to present high levels of induced defenses. For instance, Howe et al. (2020) observed that trees in general exhibit significant positive correlations between constitutive concentrations of monoterpenes and induced monoterpenes. In the study they assessed the inducibility to constitutive monoterpenes of nine species of conifers and concluded that mature trees with high constitutive monoterpene concentrations also showed high induction, although this was not the case for the assessed progenies in all the evaluated species (Howe et al. 2020).

The relationship between size of the traumatic ducts and resin yield seems to be the one that is less influenced by environmental factors given that it presented a good correlation with resin yield across ages. It was also the only anatomical trait that presented significative variation among families (*p* = 0.013 area; 0.001 diameter).

Although there seem to be an agreement that anatomical traits are essential traits for the pine tree defensive system, there have been some mixed results on which features are the most relevant as indicators of tree survival or/and high resin yield capacity. As opposed to our results, several studies have also found a positive correlation between resin flow and the number of axial resin ducts in species like *Picea abies* (L.) H. Karst., *P. nigra Aiton, P. contorta, P. flexilis, P. taeda, P. elliottii* (Rosner and Hannrup 2004; Luchi et al. 2005; Ferrenberg et al. 2014; Westbrook et al. 2015; Neis et al. 2019). On the other hand, other studies have found that radial resin ducts were positively correlated with the constitutive resin flow, for instance, Baier et al. (2002) (spring: r = 0:66; summer: r =0.54) and Rodríguez-García et al. 2014 (R^2 = 0.23) and that the rate of resin flow was strongly related to viscosity and the number and size of radial resin ducts in *P. elliottii* (Schopmeyer et al. 1954). In *Picea abies* resin flow was highly correlated with both the number and area of resin ducts (Netherer et al. 2015). Also, there was found a correlation between resin yield and volume of ducts (Rodríguez-García et al. 2014; Neis et al. 2019). Other authors did not observe any relation between anatomical traits of the resin duct system and resin yield in four southern pine species (*P. palustris* Mill.; *P. taeda* L.; *P. elliottii* Engelm; *P. echinata* Mill.) (Hodges et al. 1981).

3.5 Conclusions

The trial presented good production performance (both for resin yield and stem volume) and enough genetic variability to conduct a genetic selection for breeding. Therefore, the genetic germplasm conservation is strategic for the forestry sector, especially if we consider that many *P. oocarpa* stands are gradually being replaced by other agricultural crops, such as avocado in the state of Michoacán. The variation found indicates that it is possible to create a genetic improvement strategy for the *P. oocarpa* species at an early age aimed at improving its resin production, with significant genetic gains in the first generations of breeding. We found resin yield was under moderate genetic control, with the coefficients of genetic variation together with heritability were the largest at year four, whereas volume was under lower genetic control. It is suggested to conduct selection at family mean level at year four for resin yield and at year five for both DBH and resin yield, since the genetic correlation between resin yield and DBH was the highest at year five. The results suggest that one possible strategy in breeding for *P. oocarpa* would be to maximize resin production through selection for other additional indirect traits, as DBH and TD. The only anatomical character that presented significant genetic variation was the area and diameter of the traumatic ducts and showed a high and positive correlation with resin yield. We observed that families with larger but fewer axial ducts were more productive for resin yield, and that the ability to increase defenses in response to external challenges is also necessary to consider when selecting trees for breeding.

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DISCUSIÓN GENERAL

A pesar de que la resina es uno de los productos no maderables más importantes en México, los programas de mejoramiento genético enfocados a incrementar la producción de resina son escasos. En general, uno de los problemas asociados al mejoramiento genético de árboles forestales es el tiempo requerido para su evaluación. Reducir los tiempos de cada etapa de selección en los programas de mejora podría ser una opción para acelerar el proceso de evaluación de genotipos altamente productores.

En el presente proyecto de investigación, se evaluó la variación genética de los caracteres de crecimiento (morfología), de las estructuras de defensa (canales resiníferos en xilema y floema secundario) y el rendimiento de la producción de resina, ya que la existencia de una variación genética aditiva significativa entre familias es la condición fundamental para obtener futuras ganancias genéticas. Así mismo, a través de la genética cuantitativa se estimaron los parámetros genéticos de todas las características de nuestro interés. El objeto de estudio fueron: a) 27 familias de medio hermanos en diferentes edades (año tres, cuatro, cinco y seis) y b) 24 genotipos fenotípicamente superiores en producción de resina (árboles plus).

Además, se dio continuidad a la evaluación del método de miniresinación como una estrategia para la selección temprana de árboles superiores en producción de resina y los árboles plus seleccionados por su alta productividad se evaluaron en 48 horas. Una limitación de este estudio fue que el ensayo se realizó en un solo sitio; futuros ensayos requerirán de varios sitios de campo, para estimar la magnitud de los efectos de las variables ambientales (clima, principalmente). Sin embargo, a pesar de que los resultados de este estudio provienen de una muestra limitada, proporcionan un buen punto de referencia, sobretodo en el caso de las variables anatómicas.

Características de crecimiento**.** Los resultados del presente trabajo permitieron detectar una variabilidad genética significativa entre familias de medios hermanos en los caracteres de crecimiento (morfológicos) y ganancias genéticas de orden de 4 – 5% en la siguiente generación, a pesar de que la selección fenotípica de los árboles madre se hizo con base en su volumen de producción de resina, parece haber suficiente variación para hacer selecciones simultáneas en crecimiento y producción de resina.

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Características anatómicas. Se realizó una descripción exhaustiva de la anatomía del sistema secretor constitutivo del xilema secundario (madera) y el floema secundario (corteza) de árboles juveniles y maduros de *Pinus oocarpa*. Estudios previos han demostrado que el volumen de producción de resina entre especies varía mucho entre los árboles que son morfológicamente similares (Gary 1933; Wyman 1932), no obstante, debido a las complejas relaciones entre las estructuras secretoras y la producción de resina (Rodríguez-García et al. 2014) no se ha llegado a respuestas concluyentes sobre qué caracteres son los que están más relacionados con la producción de resina.

En ese sentido, en el presente trabajo se evaluaron la variación genética entre progenies de medios hermanos para las características de área (mm^2), diámetro (mm) y densidad (no. / mm^2) de los canales en tejidos vasculares secundarios (madera y corteza) de árboles juveniles y adultos y su relación con la producción de resina. En las características anatómicas de los árboles adultos se encontró variación significativa entre individuos para todas las características anatómicas estudiadas. Sin embargo, en las progenies se encontró variación genética significativa únicamente para la densidad de los canales axiales de xilema (no. / mm^2) (contrario a lo observado en las características de crecimiento). Esta variación entre las familias de medios hermanos indica que, de las características anatómicas constitutivas, la densidad de canales axiales es el rasgo menos influenciado por factores ambientales y que es posible esperar una mejora genética en futuras generaciones para este caracter en particular, lo cual coincide con estudios en otras especies (Westbrook et al. 2015; Govina et al. 2020). En los demás caracteres evaluados: tamaño, diámetro o número de células epiteliales tanto en los canales axiales como radiales de xilema y floema no se observaron diferencias significativas.

Así mismo, se observaron diferencias en las estructuras anatómicas de los canales resiníferos dependiendo de la edad del árbol y el tejido, lo cual sugiere que estas diferencias no son aleatorias, sino que existe cierta plasticidad fenotípica de estas estructuras relacionados a la eficacia biológica relativa (fitness) de cada uno de los tejidos vegetales en particular (Whitham 1983). Los canales radiales en general mantuvieron mayor uniformidad desde una edad temprana en ambos tejidos (xilema y floema). En general, el área de los canales aumentó con la edad y la densidad promedio de los tres tipos de canales disminuyó con la edad.

En cuanto a las estructuras relacionadas a las defensas inducidas, los canales traumáticos, presentaron variación significativa entre familias para el área y diámetro de canales.

Características de producción. La variación entre progenies de medios hermanos encontrada en la producción de resina en la producción de resina sugiere que es posible crear una estrategia de mejora genética para la especie *P. oocarpa* a una edad temprana con el objetivo de incrementar la producción de resina, lo que indica que existe la posibilidad de obtener ganancias genéticas en las primeras generaciones de mejoramiento. Las estimaciones de los parámetros genéticos sugieren que el rendimiento de la resina está bajo un control genético moderadamente alto a nivel de medias de familias (h^2_f = 0.39 a 0.54) y se pueden obtener ganancias genéticas hasta de 15-27% en la primera generación. El coeficiente de heredabilidad de medias de familia fue el más alto en el cuarto año del ensayo (h^2_f = 0.54). Los valores más altos para el rendimiento de resina sugieren que son los factores genéticos los que controlan predominantemente este carácter (Susilowati et al. 2013).

Este patrón también indica que los programas de mejora destinados a incrementar el rendimiento de resina en *P. oocarpa* pueden comenzar por una selección fenotípica de árboles individuales de alta productividad de resina (Susilowati et al. 2013), y que existe un potencial para el mejoramiento genético, dado que estudios anteriores han confirmado que es un rasgo altamente heredable (Gurgel-Garrido et al. 1996; Tadesse et al. 2001; White et al. 2007) y lo cual coincide con lo encontrado en el presente trabajo.

Así mismo, los resultados obtenidos sugieren que será posible acortar en tiempo la selección de genotipos superiores en producción de resina, ya que los coeficientes de las correlaciones edad-edad obtenidos fueron altos (r = 0.55-1.27). Estudios previos también refieren que no es necesario esperar a que los árboles tengan mayor edad para las etapas finales de selección (Squillace y Gansel 1974; Gurgel-Garrido y Kageyama 1993; Gurgel-Garrido et al.1994; Romanelli 1995; Romanelli y Sebbenn 2004; Zeng et al.2013). De las familias estudiadas, ocho familias presentaron un mayor rendimiento de resina que el promedio, similar a lo encontrado en otras especies. El volumen promedio de producción de resina de la familia de mayor rendimiento en el presente estudio superó 4.5 veces el promedio de la familia de menor rendimiento y duplicó el rendimiento promedio general. Estos datos sugieren que existe un gran potencial productivo y confirma que las progenies seleccionadas son un buen material base para un proyecto de mejoramiento genético.

Por otro lado, se encontraron evidencias que es posible acortar los tiempos de selección en la primera etapa de mejoramiento, mediante la selección de los árboles adultos con una técnica de borehole adaptado con un muestreo de 48 horas, lo cual permite acortar los costos y tiempos de selección. Esto coincide con lo encontrado en un estudio de *P. elliottii* (Neis et al. 2019). La técnica de miniresinación utilizada como un método indirecto para la selección temprana de árboles superiores en producción de resina en familias de medios hermanos en árboles juveniles, también comprobó ser una técnica viable para la selección de genotipos en ensayos de progenies de *P. oocarpa* a edad temprana. Las correlaciones edad-edad en el presente estudio también sugieren que los árboles que mostraron el mayor volumen de producción de resina en las primeras etapas tienden a repetir el rendimiento en edades más avanzadas (Pswarayi et al. 1996; Romanelli y Sebben 2004).

Rasgos anatómicos y morfológicos asociados con la síntesis de resina. Las correlaciones genéticas entre las características de crecimiento (variables indirectas) y la producción de resina en el presente estudio fueron bajas, excepto por la asociación entre el diámetro a la altura del pecho (DAP) y la producción de resina. Esta asociación moderada y positiva entre el DAP y el rendimiento de la resina muestra que los factores genéticos de ambos caracteres posiblemente actúan simultáneamente para aumentar la producción de resina y el DAP (dos-Santos et al. 2016), lo cual sugiere que individuos/familias con mayores diámetros son los más productivos o los que se adaptaron mejor. Sin embargo, hay que tener en consideración que los resultados en estudios anteriores muestran resultados mixtos sobre la correlación genética entre el rendimiento de resina y los rasgos de crecimiento. Las bajas correlaciones para la altura y el diámetro basal con la producción de resina, concuerdan con lo observado en los estudios sobre *P. elliottii* var. *elliottii* en Brasil (Gurgel-Garrido y Kageyama 1993; Romanelli 1995), *P. caribaea* (dos-Santos et al. 2016), pero son opuestos a lo que se encontró para *P. elliottii* en China, donde los rasgos de crecimiento (diámetro a la altura del pecho, altura y volumen de fuste) presentaron una correlación fuerte y positiva con el rendimiento de resina.

Anatomía *vs*. síntesis de resina. El área de los canales axiales y traumáticos mostró una correlación alta y positiva con la producción de resina, lo cual sugiere que los árboles con canales axiales de áreas de mayor tamaño podrían ser potencialmente más productivos de acuerdo con la ecuación de Hagen-Poiseuille. Según esta ley, incluso un pequeño aumento en el diámetro en los conductos capilares tiene un impacto importante en la capacidad de conducción de los canales para sustancias viscosas (Schopmeyer et al. 1954; Sperry et al. 2006). Por otro lado, la correlación negativa observada entre la frecuencia de canales (número de canales por mm^2) y la producción de resina podría sugerir que existe una posible compensación entre el tamaño y cantidad de canales en los pinos (Herms y Mattson 1992) citados por Zhao y Erbilgin (2019).

Así mismo, se observaron ductos traumáticos en el xilema secundario en zonas a una distancia de 10 cm de las heridas provocadas por la miniresinación y por la aplicación del estimulante, lo cual podría indicar una respuesta sistémica del árbol como consecuencias de este daño mecánico (Rodríguez-García 2016), ya que las muestras de madera se tomaron cuatro meses después de la última evaluación por miniresinación. La asociación encontrada entre los ductos traumáticos y la producción de resina confirma que los ductos traumáticos tienen un papel importante en el incremento de la producción de resina (Ruel et al. 1998; Lombardero et al. 2000).

CONCLUSIONES GENERALES

Los resultados obtenidos aportan nueva información respecto a las características de crecimiento y el sistema de defensas de la especie, así como sobre la producción de resina y las asociaciones entre cada una de las características evaluadas. Esta información puede ser utilizada como referencias en futuros programas de mejora de la misma especie para lograr incrementar la productividad de resina y obtener la producción de germoplasma de mayor calidad genética que se requiere para el establecimiento de las plantaciones comerciales y para la regeneración de áreas boscosas, ya que existe una creciente demanda de recursos forestales sostenibles. Los resultados sugieren que no será necesario llevar a cabo muestreos de periodos largos en la selección de árboles fenotípicamente superiores en producción de resina y que será posible acortar los tiempos de evaluación de los árboles superiores a través de sus progenies. Además, se observó que una posible estrategia para el mejoramiento de *P. oocarpa* podría ser maximizar la producción de resina mediante la selección de otros rasgos indirectos adicionales como el DAP y la evaluación de sus canales traumáticos. Según los datos obtenidos el cuarto año sería adecuado para llevar a cabo la selección de los genotipos de *P. oocarpa* en función de su potencial productivo y en el año cinco se podrá incluir el DAP en la selección, ya que el valor de heredabilidad para estos rasgos fue el más alto.

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ANEXOS

Costancias de participación en congresos

100 años UNIVERSIDAD MICHOACANA DE SAN NICOLÁS DE HIDALGO
Cuna de héroes, crisol de pensadores

PIDCB

Programa reconocido por el PNPC

CONACYT

La Universidad Michoacana de San Nicolás de Hidalgo a través del Programa Institucional de Doctorado en Ciencias Biológicas otorga la presente

CONSTANCIA A:

Irenka Fabián Plesníková, José Cruz de León, Alejandro Reyes Ramos, Cuauhtémoc Sáenz Romero, Nahum M. Sánchez Vargas, Teresa Terrazas Salgado

CON LA PONENCIA:

PARÁMETROS GENÉTICOS EN UN ENSAYO DE PROGENIES DE *Pinusoocarpa*Schiede ex Schltdl.

En la Reunión Académica 2017
del Programa Institucional de Doctorado en Ciencias Biológicas
27 y 28 de noviembre del 2017. Morelia, Michoacán.

Dr. Carlos Cortés Penagos
Coordinador del PIDCB

Dra. Rosa Elva Norma de Río Torres
Coordinadora General de Estudios de Posgrado

inirena UMSNH

FACULTAD DE MEDICINA VETERINARIA Y ZOOTECNIA

INSTITUTO DE INVESTIGACIONES AGROPECUARIAS Y FORESTALES

FACULTAD DE QUÍMICO FARMACOBIOLOGÍA

FACULTAD DE BIOLOGÍA

INSTITUTO DE INVESTIGACIONES QUÍMICO-BIOLÓGICAS

9º Foro Académico del Posgrado en Ciencias Biológicas y Agropecuarias 2018

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FACULTAD DE MEDICINA VETERINARIA Y ZOOTECNIA

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INSTITUTO DE INVESTIGACIONES QUÍMICO-BIOLÓGICAS

FACULTAD DE BIOLOGÍA

CONACYT

La Universidad Michoacana de San Nicolás de Hidalgo a través de los
Programas Institucionales de Maestría y Doctorado en Ciencias Biológicas
otorga la presente

CONSTANCIA A:

IRENKA FABIÁN-PLESNÍKOVÁ, MIGUEL MARTÍNEZ-TRUJILLO, ALEJANDRO REYES-RAMOS, CUAUHTÉMOC SAENZ ROMERO, NAHUM MODESTO SÁNCHEZ-VARGAS, TERESA TERRAZAS

POR SU PARTICIPACIÓN
CON EL TRABAJO:

Canales resiníferos y la producción de resina en Pinus oocarpa

EN EL 9° FORO ACADÉMICO DE POSGRADO EN CIENCIAS BIOLÓGICAS Y AGROPECUARIAS,
realizado los días 12 y 13 de diciembre del 2018

DRA. ROSA ELVA NORMA DEL RIO TORRES
COORDINADORA GENERAL DE ESTUDIOS DE POSGRADO

DRA. ESPERANZA MELENDEZ HERRERA
COORDINADORA GENERAL DEL PROGRAMA INSTITUCIONAL DE DOCTORADO EN CIENCIAS BIOLÓGICAS

DRA. LILIANA MÁRQUEZ BENAVIDES
COORDINADORA GENERAL DEL PROGRAMA INSTITUCIONAL DE MAESTRÍA EN CIENCIAS BIOLÓGICAS

XXVII CONGRESO NACIONAL Y VII INTERNACIONAL DE FITOGENÉTICA

BIODIVERSIDAD ANTE EL CAMBIO CLIMÁTICO

La Sociedad Mexicana de Fitogenética, A.C. y el Colegio de Postgraduados otorgan a

Irenka Fabián-Plesníková

la presente **CONSTANCIA DE PRESENTACIÓN ORAL** del trabajo

EVALUACIÓN DE DOS MÉTODOS PARA LA SELECCIÓN DE ÁRBOLES SUPERIORES EN PRODUCCIÓN DE RESINA DE *Pinus oocarpa*

Irenka Fabián-Plesníková, José Cruz-de-León, Alejandro Reyes-Ramos, Cuauhtémoc Sáenz-Romero, Nahum M. Sánchez-Vargas y Teresa Terrazas-Salgado

Durante el XXVII Congreso Nacional y VII Internacional de Fitogenética celebrado en el Campus Montecillo del Colegio de Postgraduados, en Texcoco, Estado de México, del 24 al 28 de septiembre de 2018.

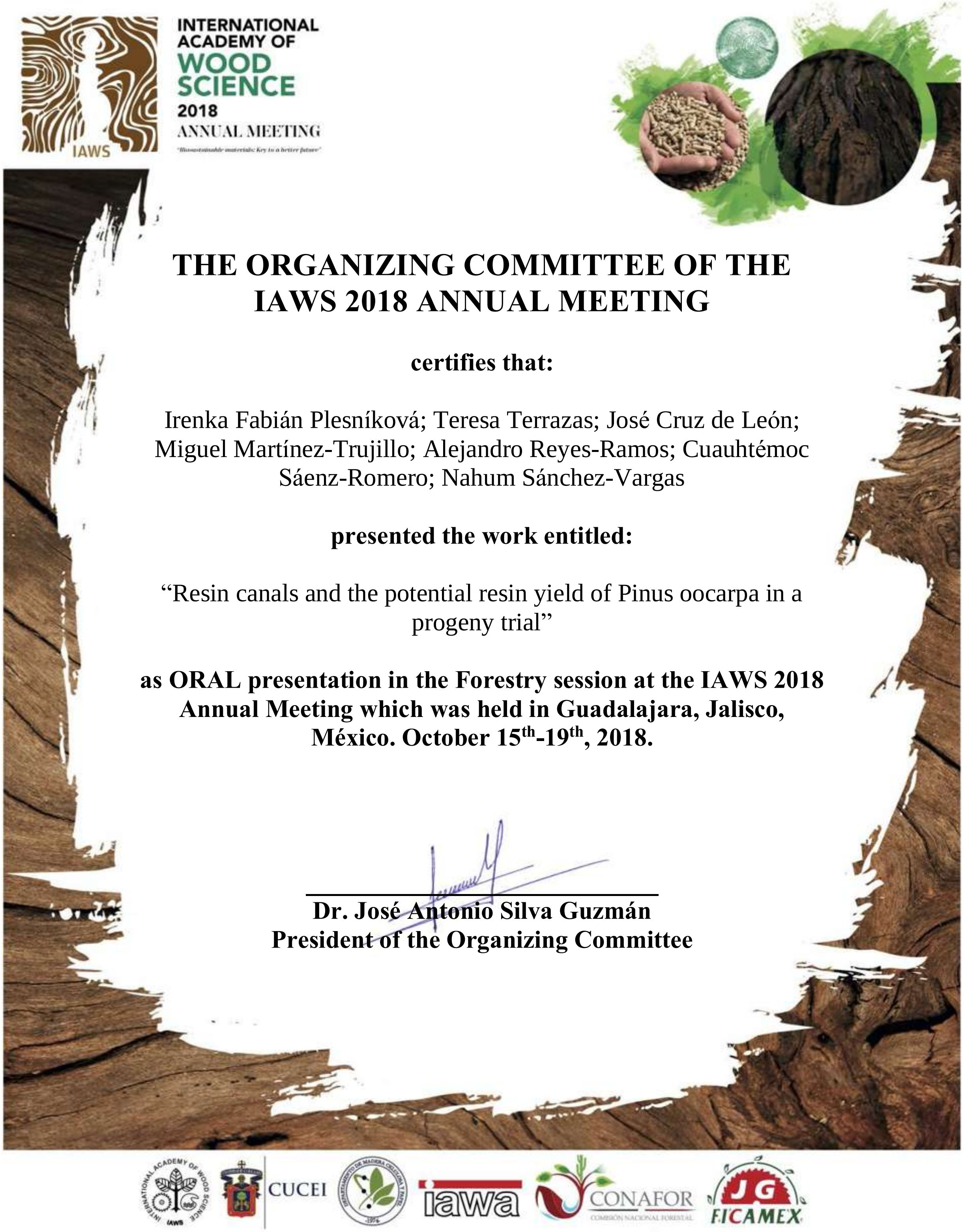
DR. RICARDO LOBATO ORTIZ
Presidente
Sociedad Mexicana de Fitogenética, A.C.

DR. BULMARO COUTIÑO ESTRADA
Secretario
Sociedad Mexicana de Fitogenética, A.C.

DRA. MARGARITA TADEO ROBLEDO
Secretaria Organizadora del Congreso
Sociedad Mexicana de Fitogenética, A.C.

DR. IGNACIO BENÍTEZ RIQUELME
Presidente del Comité Organizador Local
Colegio de Postgraduados

Id: C213B270
Fecha de expedición: 28 de septiembre del 2018
Cadena de autenticidad: d72009ca5664ef697b553e91e1699bc5639bdb43



INTERNATIONAL ACADEMY OF WOOD SCIENCE 2018 ANNUAL MEETING

THE ORGANIZING COMMITTEE OF THE IAWS 2018 ANNUAL MEETING

certifies that:

Irenka Fabián Plesníková; Teresa Terrazas; José Cruz de León; Miguel Martínez-Trujillo; Alejandro Reyes-Ramos; Cuauhtémoc Sáenz-Romero; Nahum Sánchez-Vargas

presented the work entitled:

“Resin canals and the potential resin yield of Pinus oocarpa in a progeny trial”

as ORAL presentation in the Forestry session at the IAWS 2018 Annual Meeting which was held in Guadalajara, Jalisco, México. October 15th-19th, 2018.

Dr. José Antonio Silva Guzmán
President of the Organizing Committee

EL GOBIERNO DEL ESTADO DE DURANGO,
LA UNIVERSIDAD JUÁREZ DEL ESTADO DE DURANGO,
EL CONGRESO DEL ESTADO DE DURANGO
LXVII LEGISLATURA Y EL
INSTITUTO TECNOLÓGICO DE EL SALTO

Extienden la presente

CONSTANCIA

A: Irenka Fabián Plesníková, José Cruz de León, Miguel Martínez Trujillo, Alejandro Reyes Ramos, Cuauhtémoc Sáenz Romero, Nahum M. Sánchez Vargas, Teresa Terrazas Salgado

LA PRODUCCIÓN DE RESINA Y EL MEJORAMIENTO GENÉTICO, UNA PROPUESTA HACIA EL APROVECHAMIENTO SUSTENTABLE DE LOS BOSQUES

Por la presentación de su trabajo en la modalidad cartel en el:

FORO INTERNACIONAL FORESTAL DURANGO 2018
"PASADO, PRESENTE Y FUTURO DE LOS BOSQUES TEMPLADOS DE AMÉRICA Y EUROPA"

Victoria de Durango, Dgo. Abril de 2018.

Dr. José Rosas Aispuro Torres
Gobernador Constitucional del Estado de Durango

Lic. Ricardo Fidel Pacheco Rodríguez
Presidente de la Junta de Gobierno y Concertación Política del Congreso del Estado

Profr. Dr. Christian Anton Wehenkel
Profesor-Investigador ISIMA-UJED

Mtra. Isela Flores Montenegro
Directora del ITES

La Sociedad Mexicana de Fitogenética, A.C. y la Universidad Juárez Autónoma
de Tabasco otorgan la presente **CONSTANCIA** a

Irenka Fabián Plesníková

Por haber presentado el trabajo

HEREDABILIDAD EN UN ENSAYO DE PROGENIES DE PINUS OOCARPA SCHIEDE EX SCHLTDL. PARA DOBLE PROPÓSITO

I. Fabián-Plesníková, N.M. Sánchez-Vargas, C. Sáenz-Romero, T. Terrazas-Salgado

Como **Ponencia Oral** en el XXVI Congreso Nacional y VI Internacional de Fitogenética celebrado en Villahermosa, Tabasco del 26 al 30 de septiembre de 2016.

Dr. Serafín Cruz Izquierdo
Presidente de la Sociedad

Dr. Bulmaro Coutiño Estrada
Secretario Organizador del Congreso

XXVI Congreso Nacional y
VI Internacional de Fitogenética

Dr. Roberto Flores Bello
Director de DACA-UJAT

Dr. Efraín de la Cruz Lázaro
Presidente del Comité Organizador Local

We are all connected: To each other, biologically. To the earth, chemically. To the rest of the universe, atomically. We are not figuratively, but literally stardust.

Neil deGrasse Tyson