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Articles

Seed production potential of *Pinus montezumae* Lambert in central Mexico

Potencial de producción de semilla de *Pinus montezumae* Lambert en el centro de México

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ABSTRACT

The forests that surround the cities of Mexico, Puebla and Tlaxcala are a source of environmental services for 25 million people. *Pinus montezumae* Lamb. is an abundant species in the area that is under anthropocentric pressure. The objective of the study was to determine the repopulation capacity of *Pinus montezumae* through its seed production capacity related with environmental (climate, soil) and ecological variables (tree density). In eight populations, 10 cones per tree were collected from 15 trees per population (1200 cones). Data analysis was carried out by parametric and non-parametric methods according to the behavior of each variable. Correlations were estimated using the Pearson and Spearman method. In all variables, significant differences were found ($p < 0.05$) between populations and 3 out of 11 variables between trees. On average, 132 developed seeds were obtained per cone, 55% full, 43% empty and 2% pest-ridden. Production efficiency, reproductive efficiency and endogamy index were 0.33, 28.7 and 0.43, respectively. Some seasonal climatic factors showed associations with seed production. A lower seed production potential ($r < -0.70$) was observed in populations with high temperature and low precipitation and a higher seed weight when the average temperature increased ($r = 0.74$). Organic matter has a negative influence on seed weight ($r = -0.94$), while more acidic soils have greater potential for seed production ($r = 0.76$). Stand density did not show any significant correlation ($p < 0.05$; $r < \pm0.26$). Despite the high fragmentation and low stand density in the populations, the reproductive indicators do not show a deficiency in the production of full seed, which indicates a correct natural regeneration process by seed. The substantial increase in temperature and reduction in precipitation could reduce the species' seed production and its ability to repopulate naturally. This warns of the need for adequate management of forests under an unfavorable climate scenario, and one that is focused on the renewal of populations through reforestation programs.

Keywords: Endogamy; Reproduction efficiency; Reproductive indicators; Seed weight

RESUMEN

Los bosques que rodean las ciudades de México, Puebla y Tlaxcala son fuente de servicios ambientales para 25 millones de personas. *Pinus montezumae* Lambert es una especie abundante en la zona que está bajo presión antropocéntrica. El objetivo del estudio fue determinar la capacidad de repoblamiento a través del análisis de indicadores reproductivos y su relación con 26 variables ambientales (clima, suelo) y ecológicas (densidad de árboles). En ocho poblaciones se recolectaron 10 conos sanos por árbol, de 15 árboles por población (1200 conos). Los datos se analizaron con las pruebas GLIMIX y Kruskal–Wallis, según el comportamiento de cada variable. Las correlaciones se estimaron por el método de Pearson y Spearman. Se encontraron diferencias significativas ($p < 0.05$) en todas las variables entre poblaciones y en 3 de 11 variables entre árboles. Se obtuvo un promedio 132 semillas desarrolladas por cono, 55% de ellas se encontraban llenas, 43% vanas y 2% plagadas. La eficiencia de producción, eficiencia reproductiva y el índice de endogamia fueron 0.33, 28.7 y 0.43, respectivamente. Algunas variables ambientales mostraron asociaciones con la producción de semilla. Se observó menor potencial de producción de semilla ($r < -0.70$) en poblaciones con elevada temperatura y baja precipitación, además de mayor peso de semilla al aumentar la temperatura media ($r = 0.74$). El peso de semilla disminuye a menor disponibilidad de materia orgánica ($r = -0.94$), mientras que suelos más ácidos poseen mayor potencial de producción de semilla ($r = 0.76$). La densidad no presentó correlación significativa con ninguna variable ($p < 0.05$; $r < \pm0.26$). A pesar de la alta fragmentación y baja densidad de árboles en las poblaciones, los indicadores reproductivos no muestran deficiencia en la producción de semilla llena, siendo indicio de un correcto proceso de regeneración natural por semilla. El aumento sustancial de la temperatura y la reducción de la precipitación podría reducir la producción de semilla de la especie y su capacidad de repoblarse naturalmente. Esto advierte la necesidad de un manejo adecuado de los bosques bajo un escenario climático desfavorable, enfocado a la renovación de las poblaciones por medio de programas de reforestación.

Palabras clave: Endogamia; Eficiencia de producción; Indicadores reproductivos; Peso de la semilla

1 INTRODUCTION

The natural regeneration of the forest depends on the capacity to produce viable seed, in addition to competition, predation and water availability that favor the establishment and development of new individuals. The climatic (temperature, precipitation, light), genetic (genotype of the individual and its origin) and ecological (tree density, competition) conditions of the site have a direct effect on seed production (FERNANDO, 2013). Differences in seed availability between populations for a production cycle have been observed due to geographic and climatic differentiation along with interannual variations in temperature and precipitation (CAPILLA DINORI *et al*., 2021). The genetic constitution of the tree and environmental changes are some of the causes of variation in seed production among populations, probably as an adaptive mechanism (LUCAS BORJA and VACCHIANO, 2018).

The seed production of a species is an indicator that helps determine its ability to remain at a given site, and its reproductive efficiency can be determined by evaluating seed production per tree, the proportion of aborted ovules, and the ratio of full to empty seeds (QUIROZ VÁZQUEZ *et al*., 2017). Endogamy is a product of self-fertilization or crossbreeding of genetically related individuals that causes elimination of the megagametophyte (TAKEUCHI *et al*., 2020); thus, empty seed is a measure of endogamy.

Seed production potential has been evaluated in seed orchards and in natural pine stands and differences between species and provenances have been found (SANTOS SÁNCHEZ *et al*., 2018). However, it is not common to evaluate the relationship between seed production and environmental variables in species vulnerable to land use change. When the latter come to be considered, it is common that annual averages of precipitation and temperature are used (CAPILLA DINORI *et al*., 2021). There is only one report on the seed production capacity or reproductive indicators of *Pinus montezumae* Lambert (DELGADO VALERIO, 1994) despite being a species valued for the quality of its wood and its ecological importance for its contribution to environmental services to the surrounding populations (FLORES GARCÍA and MOCTEZUMA LÓPEZ, 2021). Currently, the continuous disturbance of forests is evident, mainly due to agricultural activity. The decrease in tree density could result in less natural regeneration due to the lack of pollen from adult trees.

In Mexico, there are few studies on reproductive indicators for species of the genus *Pinus*, so it is necessary to develop studies to generate solutions for areas under anthropocentric pressure. The objective of this study was to determine the natural regeneration capacity of *Pinus montezumae* through the analysis of reproductive indicators and their relationship with environmental variables.

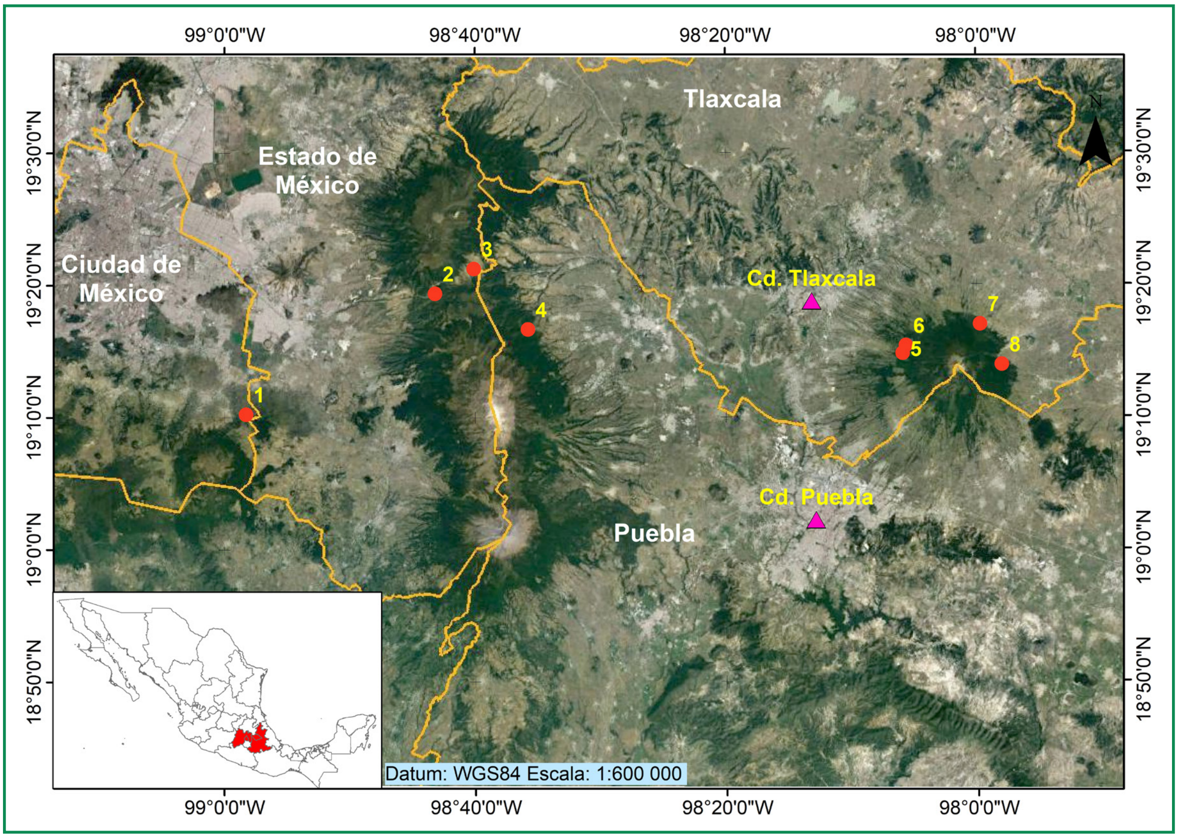
2 MATERIALS AND METHODS

2.1 Cone collection

The cones were collected from eight populations distributed in the states of Mexico, Puebla, Tlaxcala and Mexico City (Central Mexico; Table 1; Figure 1). The collection was carried out during the month of December 2021, when the cones had matured. One day was used for each population; the order from East to West was followed.

In each population, 15 healthy adult trees were selected with a minimum distance of 50 meters between them to obtain the greatest variation within populations (BRAMLETT *et al*., 1977). The density of *Pinus montezumae* trees was determined in order to relate it to seed production. Circular sites with a radius of 17.84 m were surveyed.

Figure 1 – Distribution of *Pinus montezumae* populations under study



Source: Authors (2022)

In where: Names of the populations in Table 1.

Table 1 – Location and climatic data of the eight *Pinus montezumae* populations under study

Population	Latitude	Longitude	Altitude (MASL)	Precipitation (mm)	Temperature (°C)	Organic matter	pH	Density
Tlacotenco	19°10.80′	98°36.90′	2959	901 (826)	13 (3.4)	10.5	5.4	9.0
Llano Grande	19°20.83′	98°43.53′	3176	883 (807)	11 (1.1)	7.1	5.4	10.7
Tlahuapan	19°20.90′	98°39.35′	2844	912 (827)	13 (1.9)	6.6	5.3	6.2
San Rafael	19°16.8′	98°35.85′	2913	928 (849)	13 (3.7)	6.9	5.3	15.8
San Pedro	19°15.56′	98°05.08′	2949	910 (794)	14 (8.9)	4.8	4.4	10.2
San Bartolo	19°16.30′	98°05.53′	2850	896 (775)	14 (10.0)	3.3	4.4	19.3
Altamira	19°16.96′	97°59.45′	3000	912 (839)	13 (8.0)	6.7	3.5	22.8
Ixtenco	19°17.41′	97°58.21′	2950	904 (825)	14 (9.4)	3.5	3.6	18.1

Source: Authors (2022)

In where: The precipitation column indicates the annual mean, and the fall average is in parenthesis. The temperature column indicates the annual mean; the minimum for winter is in parenthesis. The annual means for precipitation and temperature are averages for the years 2018, 2019, and 2020 using the ClimateNA 6.4 software.

Healthy cones were chosen, without deformations and without holes on the outside caused by insects. Ten closed cones with physiological maturity were collected from each tree and stored in paper bags with population nomenclature, tree and cone number. The cones were dried under shade at an average daytime temperature of 28°C for ten days. They were then kept for another two weeks in greenhouse conditions where they opened completely. The average daytime temperature was 35°C.

The cones were sectioned with a common drill; a hole was made at the base of the cone to separate and count the fertile scales and facilitate the extraction of full, empty, pest-ridden and aborted ovules. The sum of the first three were developed seeds (those that were of normal size, which is greater than 4 mm); those of smaller size were classified as aborted ovules (BRAMLETT *et al*., 1977). Pest-ridden seeds were identified by the presence of a hole in the testa. To identify full and empty seeds, they were separated by flotation in water and alcohol (10 %) for five minutes. The testa was broken and the presence or absence of the megagametophyte was observed as validation. On an electronic scale accurate to 100 µg, the mass of full seeds per cone was obtained to determine the weight of 100 seeds. Finally, the cones were dried in an oven at 72°C for 72 h (MOSSELER *et al*., 2000) to obtain their dry weight (g).

The following seed production variables were obtained (BRAMLETT *et al.*, 1977), from the equations:

Seed Production Potential (SPP) = Fertile scales * 2 = total fertile ovules (1)

Developed seeds (DS) = full seeds + empty seeds + pest-ridden seeds (2)

Percentage of developed seed (PDS) = (developed seeds / seed potential)*100 (3)

Percentage of aborted ovules (PAO) = (aborted ovules/seed potential)*100 (4)

Percentage of full seeds (PFS) = (full seeds/developed seeds)*100 (5)

Percentage of empty seeds (PES) = (empty seeds/developed seeds)*100 (6)

Percentage of pest-ridden seeds (PPS) = (pest-ridden seeds / developed seeds)*100 (7)

Weight of 100 seeds (WHS) = average weight of a seed*100 (8)

In addition, the following reproductive indicators were calculated from the equations below:

Seed production efficiency (SPE) = full seed /SPP (9)

Reproductive efficiency (RE) = (weight of full seeds / dry weight of the cone)*100 (10)

Endogamy index (EI) = empty seeds / developed seeds (11)

With the above variables, the global fertilization value of the ratio between developed seeds and total ovules was determined. In addition, the fertilization threshold was identified — a value that is the minimum percentage of developed seed per cone (BROWN, 1971).

2.2 Soil analysis

A subsample of soil was collected in the first 10-20 cm of depth and no more than 3 meters from the tree. The soil was uncovered by removing the layer of woody material and leaf litter. The subsamples were mixed to form a 1 kg soil sample per population. These were analyzed at the Laboratorio Nacional de Investigación y Servicio Agroalimentario y Forestal (LANISAF), which is located at the Universidad Autónoma Chapingo, Texcoco, Mexico. The pH was determined with a 1:2 ratio; ammonium (NH_4) and nitrate (NO_3) concentration with a flow injection analyzer, phosphorus with an ultraviolet-visible spectrophotometer and organic matter with the Walker and Black method (LANISAF, 2021).

2.3 Statistical analysis

Seed production was analyzed using the following model in Equation (12):

$$Y_{ijk} = \mu + P_i + A_{j(i)} + e_{ijk} \tag{12}$$

Where: Y_{ijk}= response variable; μ = overall mean; P_i = fixed effect of the i-th population; $A_{j(i)}$ = random effect of the j-th tree of the i-th population; and e_{ijk} = data experimental error per cone.

Variance components were estimated with the VARCOMP procedure. Production potential, number of seeds developed and the weight of 100 seeds showed normal distribution of standardized residuals. These variables were analyzed with the SAS 9.4 statistical analysis package. The GLIMMIX procedure (generalized linear mixed model) was used to evaluate the significance of the population and tree factors using a P value equal to 0.05. Means were contrasted using the LSMEANS option with Tukey-Kramer adjustment. Non-parametric tests were used for the percentage of developed, full, empty, and pest-ridden seeds, aborted ovules, and production efficiency, reproductive efficiency and endogamy efficiency indexes since they did not show normal distribution of the standardized residuals. The significance level of the population factor was analyzed using the Kruskal-Wallis rank method. The medians were contrasted with Dunn's test for multiple comparisons with Bonferroni adjustment. The statistical package R.4.0.3 (GOSZKA and SNELL, 2020) was used in all analyses.

Climate variables were calculated from the mean elevation, latitude and longitude of each population using ClimateNA 6.4 software (WANG *et al*., 2016). Annual and seasonal mean values were considered for 2018, 2019, and 2020 — the years where climate should have influenced seed development from differentiation to maturation (PARKER *et al*., 2013). Four annual and 16 seasonal variables were used with one climate datum per population. Population means of seed variables were correlated with edaphic, climatic and tree density variables of *Pinus montezumae*. Pearson's method was used for variables with normal distribution and Spearman's method for those with non-normal distribution. The analysis was performed in R.4.0.3 (GOSZKA and SNELL, 2020).

3 RESULTS

Significant differences ($p < 0.05$) were found in all variables analyzed by parametric methods at the population and tree level. Most of the variation was due to differences between trees and error (Table 2).

Table 2 – Variance components of *Pinus montezumae* reproductive variables from eight populations in central Mexico

Variable	Total Variance	Variance components (%)		
		Population	Tree	Error
Production potential	1762.66	9.97 *	56.73 *	33.30
Developed seeds	2780.48	17.96 *	54.70 *	27.34
Percentage of developed seeds	409.96	14.52 *	55.81	29.68
Percentage of full seeds	0.7698	0.85 *	63.53	35.63

To be continued ...

Table 2 – Conclusion

Variable	Total Variance	Variance components (%)		
		Population	Tree	Error
Percentage of empty seeds	0.4057	1.31 *	64.69	34.01
Percentage of pest-ridden seeds	1.2020	24.51 *	27.68	47.82
Percentage of aborted ovules	0.3377	15.15 *	49.09	35.77
Weight of 100 seeds	0.3655	5.20 *	46.43 *	48.37
Seed production efficiency	0.9015	3.85 *	62.01	34.14
Reproductive efficiency	0.4094	0.34 *	66.01	33.65
Endogamy index	0.4057	1.31 *	64.69	34.01
Average	-	8.2	56.2	35.6

Sources: Authors (2022)

In where: *Variables with significant difference for the population and tree factors ($p \leq 0.05$) according to Tukey-Kramer and Dunn's tests.

The SPP showed a range of variation of 47 seeds between populations (Table 3) and between trees of 282 seeds (min. 120 - max. 402). Aborted ovules (AO) accounted for 42% and developed seeds (DS) for 58% of the SPP (95 AO and 132 DS). DS showed the overall fertilization value to be 0.58. The PDS among trees ranged from 11-90% of the SPP. PAO was greater than 90% of SPP in 9 cones out of 1100 evaluated. More than half of the cones showed a PAO higher than 30%. The PFS represented 57% (75/132) of the DS. Between trees, a minimum of 1 and a maximum of 189 filled seeds per cone were observed. In more than half of the cones the PFS is higher than 55% of the SPP. The PES represented 43% of the DS, but the variation between trees is from 3 to 99%. The average PPS was 2% of the DS, the inter-tree variation was zero and 21% (0-48 pest-ridden seeds). Seventy-nine percent of the cones sampled had no pest-ridden seeds. In the weight of one hundred full seeds (WHS), the variation among trees was 1.12-3.31 g. In 56% of the trees, the average value was higher than 2g.

Table 3 – Population means (± standard error) and extreme values of seed production variables among trees from eight *Pinus montezumae* populations

Population	Production Potential	Production potential %		Developed seed %			Weight of 100 seeds
		Aborted ovules	Developed seed	Full	Empty	Pest-ridden	
Tlacotenco	231 abc (± 4) 163-343	31 de (± 1) 22-66	69 ab (± 1.3) 34-78	63 a (± 1.6) 28-85	37 c (± 1.6) 14-71	0 c (± 0.1) 0-2	1.7 b ± 0.03 1.2-2.5
Llano Grande	233 abc (± 3) 170-279	46 bc (± 1.5) 19-72	54 c (± 1.5) 27-81	51 bc (± 2) 1-80	49 ab (± 2) 20-99	0 c (± 0.1) 0-2	2.1 a ± 0.03 1.5-2.8
Tlahuapan	238 ab (± 2) 208-271	31 de (± 1.5) 20-66	69 ab (± 1.5) 34-80	47 c (± 1.8) 15-78	50 a (± 2) 15-84	3 b (± 0.6) 0-16	2.1 a ± 0.05 1.2-3.1
San Rafael	248 a (± 3) 200-306	41 cd (± 1) 21-59	59 b (± 1.2) 41-79	57 ab (± 2) 11-89	41 c (± 2) 11-89	2 b (± 0.4) 0-15	1.9 a ± 0.04 1.2-2.7
San Pedro	201 c (± 4) 137-289	57 a (± 1.5) 32-77	43 d (± 1.5) 23-68	59 ab (± 1.5) 32-80	41 bc (± 1.5) 20-68	0 c (± 0.1) 0-1	2.1 a ± 0.04 1.2-3.2
San Bartolo	210 bc (± 3) 180-289	50 ab (± 1.6) 23-89	50 cd (± 1.7) 11-77	49 c (± 1.7) 18-79	50 a (± 1.7) 21-82	1 b (± 0.2) 0-7	2.2 a ± 0.07 1.1-3.1
Altamira	217 abc (± 3) 183-253	33 de (± 2) 10-74	67 ab (± 2) 26-90	52 bc (± 2.3) 7-78	40 c (± 2.3) 20-92	8 a (± 1) 1-21	2.0 a ± 0.04 1.8-2.3
Ixtenco	216 abc (± 3) 196-254	28 e (± 2) 10-66	72 a (± 2) 34-90	63 a (± 1.8) 36-97	36 c (± 1.8) 3-64	1 b (± 0.3) 0-5	2.1 a ± 0.06 1.4-3.3
Average	227	42	58	55	43	2	2.02

Source: Authors (2022)

In where: *Mean values with different letters in the columns indicate significant differences ($p \leq 0.05$) according to Tukey-Kramer and Dunn's tests. The ranges of variation in the populations correspond to the extreme values of trees within the populations.

SPE showed a contrasting variation among trees from 0.04 to 0.86 (Table 4). In RE, the average was 28.7 mg seed and with a variation among trees from 0 to 88 mg/g cone. The variation of the endogamy index was from 0 to 0.99 among trees, which means that 32 % of the trees had an endogamy index higher than 0.5.

Table 4 – Population medians (± standard error) and extreme values of trees in the reproductive indicators of eight *Pinus montezumae* populations

Population	Production efficiency	Reproductive efficiency	Endogamy index
Tlacotenco	0.38 ± 0.015 a 0.21-0.61	29 ± 1.1 a 11-55	0.31 ± 0.02 c 0.14-0.71
Llano Grande	0.25 ± 0.015 bc 0.04-0.64	30 ± 1.7 a 0-58	0.42 ± 0.02 ab 0.19-0.99
Tlahuapan	0.28 ± 0.015 b 0.07-0.60	29 ± 1.7 a 4-88	0.51 ± 0.02 a 0.15-0.84
San Rafael	0.34 ± 0.015 ab 0.06-0.62	31 ± 1.5 a 5-60	0.31 ± 0.02 bc 0.10-0.88
San Pedro	0.24 ± 0.012 bc 0.09-0.54	22 ± 1.0 bc 8-40	0.37 ± 0.02 b 0.19-0.67
San Bartolo	0.23 ± 0.011 c 0.07-0.47	19 ± 1.0 c 9-40	0.50 ± 0.02 a 0.21-0.81
Altamira	0.31 ± 0.02 ab 0.02-0.70	26 ± 1.4 ab 2-44	0.34 ± 0.02 bc 0.19-0.92
Ixtenco	0.40 ± 0.02 a 0.21-0.86	32 ± 1.4 a 17-52	0.35 ± 0.02 bc 0.01-0.63
Average	0.33	28.7	0.43

Source: Authors (2022)

In where: *Mean values with different letters indicate significant differences ($p \leq 0.05$) according to Dunn’s test. The ranges of variation in the populations correspond to the extreme values of trees within the populations.

SPP was most strongly related to climatic and soil variables. Relative humidity (r = -0.78), mean annual (r = -0.71) and winter (r = -0.86) temperatures were negatively associated with seed production potential. Autumn precipitation showed a positive relationship with PDS and negative with PAO (r = ±0.75). The number of empty seed was negatively associated with relative humidity (r = -0.64). Weight of 100 seeds was related to mean annual temperature (r = 0.74) and organic matter content (r = -0.94; Table 5).

Table 5 – Correlation values between environmental variables and reproductive indicators of eight *Pinus montezumae* populations.

	PPS	POA	PSD	PSL	PSV	PSP	WHS
DENSITY	-0.02	0.16	-0.16	0.16	-0.25	0.12	0.07
P	-0.16	0.07	-0.07	-0.01	-0.24	0.41	0.42

Table 5 – Conclusion

	PPS	POA	PSD	PSL	PSV	PSP	WHS
NH_4	0.06	-0.08	0.08	0.01	-0.31	0.57	-0.52
NO_3	**0.81***	-0.37	0.37	-0.15	0.27	-0.19	0.44
O.M.	0.63	-0.34	0.34	0.18	0.20	0.03	**-0.94***
pH	**0.76***	0.04	-0.04	-0.09	0.37	-0.48	-0.54
PPFall	0.63	**-0.75***	**0.75***	0.17	-0.47	0.48	-0.54
RHumdity	**-0.78***	0.27	-0.27	0.42	**-0.64***	0.10	0.34
MATemp	**-0.71***	0.10	-0.10	0.29	-0.36	-0.01	**0.74***
WMinTemp	**-0.86***	0.29	-0.29	-0.20	-0.31	0.12	0.51

Source: Authors (2022)

In where: DENSITY: tree density; P: available phosphorus; NH4: ammonium; NO3: nitrate; O.M.: organic matter; pH: potential hydrogen; PPFall: precipitation in fall; RHumidity: relative humidity; MATemp: mean annual temperature; WMinTemp: winter minimum temperature. Correlation significance: *p < 0.05. N=8.

4 DISCUSSIONS

The population factor contributes little to the variance of reproductive indicators in *Pinus montezumae* compared to other conifers such as *Pinus cooperi* C.E. Blanco and *Pinus durangensis* Martínez where populations contributed 60% and 48% of the variation, respectively (MARTÍNEZ RIVAS *et al*., 2020); in *Pinus chiapensis* (Martínez) Andresen, a contribution of 36-58% was observed, except in full, empty and pest-ridden seeds (CAPILLA DINORI *et al*., 2021). In *Pinus montezumae,* more than 50% of the variance is due to the variation among trees in all variables, except in the number of pest-ridden seeds.

The high contribution of trees to the total variance shows that it is convenient to emphasize individual characteristics during germplasm collection in this *Pinus montezumae* distribution zone. On the other hand, the low contribution of the population factor to the total variance suggests considering populations as the only source, where germplasm can be freely used in reforestation programs although a detailed analysis is needed to define the need for zoning of germplasm use in central Mexico. It will be necessary to define the zoning of germplasm use and identify genetic differences with molecular markers (ZHANG *et al*. 2020) or with common garden tests to define phenotypic plasticity (SIMENTAL RODRÍGUEZ *et al*. 2021).

4.1 Seed production variables

The average SPP was slightly higher than that reported for *Pinus montezumae* in the state of Michoacán (219) (DELGADO VALERIO, 1994). Ovule abortion is the main factor in seed loss caused by homozygosis of lethal genes (BRAMLETT *et al*., 1977). Pollen absence has an ontological and ecological origin affecting mainly free-crossing species that reject self-pollination (GOUBITZ *et al*., 2002). The observed fertilization failure (42% aborted ovules) could be considered as an acceptable level for natural conifer populations as the fertilization value of 0.58 is lower than that reported in *Pinus albicaulis* Engelm., which was 0.84 (OWENS *et al*., 2008). Fertilization success depends on tree (GOUBITZ *et al*., 2002), stand (FERNANDO, 2013) and climate (TIMERMAN and BARRETT, 2019) characteristics. In pines, the removal of strobili has been observed when the ovule fertilization value is low; there is a threshold value for fertilization (BROWN, 1971). This process saves energy because if they were to reach maturity, the energy invested in the cone would be greater than that devoted to the seeds (GOUBITZ *et al*., 2002). In *Pinus halepensis* Mill. cones are eliminated when the number of fertilized ovules does not exceed 30% (GOUBITZ *et al*., 2002). In *Pinus montezumae*, a threshold value could not be defined since cones continue to grow even with full seed (0.50% of the SPP).

Insect damage was low, so it does not represent a problem in seed production. In natural stands, it is common to observe insect damage. Delgado Valerio (1994) reported 0.10% insect damage, and Owens *et al*. (2008) reported 1.50% in *Pinus albicaulis*. When damage is greater, it indicates a significant pest problem as reported in *Pinus cooperi* and *Pinus durangensis,* where seed loss by insects was 49 and 52 %, respectively (MARTÍNEZ RIVAS *et al*., 2020).

The mean SPE of 0.33 is higher than that reported in natural pine stands with low density of adult trees as in *Pinus leiophylla* Schiede ex Schltdl. *et* Cham. with an SPE of 0.02 (MORALES VELÁZQUEZ *et al*., 2010) or in comparison to young stands of *Pinus sylvestris* L. where the value was 0.18 (SIVACIOGLU and AYAN, 2008). However, the SPE of the present study is lower than that reported in mature natural stands with little disturbance such as in *Pinus pinea* L. where the SPE was 0.90 (GANATSAS *et al*., 2008) and 0.59 in *Pinus albicaulis* (OWENS *et al*., 2008).

Although the SPE is relatively high, it is necessary to evaluate whether the current production of viable seed is sufficient to continue the natural regeneration cycle. Little regeneration was observed during harvesting, although this could be due to disturbance factors. The average RE indicates that there are 28.70 mg of seed per g of cone. This value represents the ratio between the energy contained in the seeds and in the cones (GÓMEZ JIMÉNEZ *et al*., 2010). The mean was higher than the 2.49 reported for *Pinus leiophylla* (MORALES VELÁZQUEZ *et al*., 2010).

The endogamy index suggests that 43 % of the seed was the product of self-pollination or by related individuals (TAKEUCHI *et al*., 2020). Natural populations under pollen-limiting conditions experience greater endogamy. For example, in Mexico, *Pseudotsuga menziesii* (Mirb.) Franco is a species with small, fragmented and low-density populations where the endogamy index is 0.81 (MÁPULA LARRETA *et al*., 2007). Endogamy is at an acceptable level despite the disturbance probably because this species forms pure stands and shows dominance. For example, in small and distant populations with over-mature *Picea rubens* Sarg. trees, a relatively low endogamy value (0.38) was reported (MOSSELER *et al*., 2000).

Age is important in seed production since in young or over-mature stands, the cones show few full seed. In young trees, the reproductive capacity may be affected by physiological immaturity. For example, a 13-year-old seed orchard of *Pinus sylvestris* (which reaches maturity at 20 years) had an endogamy index of 0.56 (SIVACIOGLU and AYAN, 2008). In Tlacotenco, despite having selected individuals of greater maturity, endogamy was lower than in the other populations evaluated in this study. Endogamy is lower when environmental conditions favor cross-pollination — mainly the high density of individuals. In a *Pinus pinea* population with a high density of mature trees (25 years old), the endogamy value was 0.05 (GANATSAS *et al*., 2008). In mature seed orchards, the same would occur due to the optimal density and efficient distribution of unrelated clones.

4.2 Relationship between reproductive indicators and climatic and soil variables

The relationship between SPP and pH is due to the fact that in pine forests, the pH is 5 to 7, which makes more acidic soils less favorable (RZEDOWSKI, 2006). The relationship with nitrate may be because this compound is necessary in the development of plant structures such as cones (AGBESHIE and ABUGRE, 2021) in the same way organic matter improves the physical and chemical properties of the soil. However, a negative relationship was observed between seed weight and organic matter content. Perhaps the relationship is explained as an adaptation to poor soils where large seeds would give greater advantage for seedlings to establish such as occurs with adaptation to water deficit (CALAMA *et al*., 2017). In addition, the analysis shows that both decreased precipitation and increased temperature cause negative effects on SPP. Both conditions could cause decreased photosynthesis and water stress in trees. In *Pinus halepensis* and *Pinus koraiensis* Siebold & Zucc., precipitation influences full seed ($r > 0.95$), so it is possible to predict production (KIM *et al*., 2020).

The empty seed showed no relationship with tree density although in pines, it has been observed that this is the factor with the greatest influence on the empty seed, followed by the age of the individuals and the stand structure (FERNANDO, 2013). Also in pines, more empty seed has been observed when the environmental humidity is low; this is because the pollen sacs open earlier when the environment is dry (TIMERMAN and BARRETT, 2019). In the evaluated populations of *Pinus montezumae*, an opposite effect was observed. In this case, in humid environments, the caking of pollen would be caused, which would hinder its dispersal.

The increase in temperature in *Pinus palustris* Mill. caused an increase in the number of male cones with respect to female cones (GUO *et al*., 2013). In *Pinus montezumae,* temperature showed a negative effect on SPP possibly due to an adverse effect on photosynthesis by generating water stress — finally reflected in cone development. Seed weight was observed to be lower in cold areas probably due to the difference in the length of the growth period, which is normally shorter in areas of low temperature as in *Pinus banksiana* Lambert and *Pinus halepensis*, where the seed weight of cold origins is lower (THANOS, 2000).

Also, weight variation could be caused by local adaptation related to a mechanism of resistance to water stress caused by temperature. Populations with higher temperatures, where greater stress is experienced, produce large seed that generate vigorous seedlings with the capacity to resist water deficit (CALAMA *et al*., 2017). *Pinus montezumae* populations located in Guatemala, which has a higher mean temperature due to lower latitude, produce larger seed (HERNÁNDEZ MOLINA, 2004). In addition, seed weight can be influenced by tree age as in *Pinus pinea*, where older stands produce smaller and lighter seed (GANATSAS *et al*., 2008). In Tlacotenco, lower seed weight was obtained probably due to the selection of individuals that showed greater maturity.

The conifer and oak forests that surround Mexico City and the metropolitan area of Tlaxcala and Puebla represent the main source of environmental services where nearly 25 million people live. The Mexican government has restrictions on commercial use in certain areas, so repopulation relies entirely on natural regeneration. *Pinus montezumae* is one of the main species growing in these areas and despite the fragmentation of its populations due to anthropocentric pressure from land use change and illegal logging, it was observed that it has the capacity to repopulate due to adequate production of full seed.

5 CONCLUSIONS

The evaluated populations of *Pinus montezumae* currently do not present problems in the production of full seed, which shows that the regeneration capacity of these populations is maintained at an acceptable level. However, it would be important to evaluate the reality of repopulation, the number of new individuals and/or their survival within the populations in subsequent studies.

Soil nitrate concentration has a positive relationship with seed production potential. This fact can be favorable in seed orchards that intend to have a large number of seed.

In spite of the fact that the evaluated populations of *Pinus montezumae* show a good regeneration capacity due to the number of full seed, it is shown that the substantial increase in temperature and decrease in precipitation implies lower seed production, but an increase in seed weight. With this, the regeneration capacity is compromised, so it is necessary to carry out further evaluations to assess the risks involved in these environmental changes due to global warming. In addition, this warns of the need for adequate forest management under an unfavorable climate scenario, which is focused on the renewal of populations through reforestation programs.

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