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SEED PRODUCTION AND QUALITY OF *PINUS DURANGENSIS* MART., FROM SEED AREAS AND A SEED STAND IN DURANGO, MEXICO

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Abstract

Seed productive potential, production efficiency and seed quality of seed areas of *P. durangensis* Mart. from La Florida and La Campana, and from a Pericos seed stand, located in Durango state, Mexico were investigated. The productive potential, developed seeds, upper and lower infertile ovules, and aborted ovules during the first and second year of seed formation were determined. X-ray scanning was used to determine the percentage of seeds that were filled, emptied, malformed, or damaged by insects. Seed production efficiency was also determined. Speed, value and percentage of germination were determined under laboratory conditions. The Pericos seed stand had the greatest seed productive potential, in terms of number of developed seeds per cone (100.3) and filled seeds (79.7), and in the percentage of filled seeds (73.4%) and seed germination (53.8%). The Pericos seed stand had the highest seed production efficiency (57.6%); this low efficiency reflects problems of damage caused by insects and deficient management. The highest ovule abortion during the first year was observed at La Campana seed area, due to self-pollination or damage by the seedbug *Leptoglossus occidentalis* in the early stages of seed development.

Introduction

Propagation of individuals through seed allows continuity in nature and promotes genetic diversity (Rauf *et al.*, 1985; Blake *et al.*, 1989). Simultaneously seeds have significant effect on plant population regeneration and plant distributing area expansion (Lv *et al*., 2012). Diversity contributes to the preservation and evolution of species; additionally, diversity influences the development of programmes for genetic improvement and commercial forestry plantations (Zobel & Talbert, 1988; Narváez, 2000; Prieto & López, 2006; Espinoza *et al*., 2009). The success of plantations is related to several factors; one of the most important is the quality of genetic germplasm (Martínez *et al*., 1994).

In Mexico, there are important programmes of plant production for reforestation and restoration of degraded areas. The annual average reforested area between 1993 and 2009 was of 2,861 ha, which equates to a production of 3,857,904 plants annually (SEMARNAT, 2011a; SEMARNAT, 2011b) and requires the use of a huge amount of seed. However, during restoration, plantations barely exceed a seedlings survival rate of 54% a year after planting (Colegio de Posgraduados, 2008). Thus, there is a need to improve factors related in the production process, going from germplasm to plantation.

Germplasm quality influences the speed and percentage of germination, as well as the growth of seedlings (Bustamante-García *et al.,* 2012a). During the process of reproduction, mainly at the flowering and fruiting stages, the seed can be affected by external factors such as the weather, birds, mammals, insects, fungi and bacteria (Anon., 1991; Mápula *et al*., 2006). Soil and nutritional factors also influence the fecundity of plants (Besnier, 1989). Additionally, inbreeding in populations with a history of exogamy affects seed production, reducing the germination capacity, the initial growth and the survival of seedlings (Sorensen & Campbell, 1993; Hart & Clark, 2007; Velasco *et al*., 2007).

Characterization of seed batches is based on analysis of the morphological and nutritional properties of fruits and seeds (Bonner, 1993). With the aim of selecting the most productive trees and predicting the production of fruits and seeds, some authors consider that the evaluation of potential seed and production seed production efficiency allows to quantify the individual production of trees (Bramlett *et al*., 1977; González *et al*., 2006; Mendizábal-Hernández *et al*., 2010).

Seed stands, seed areas and seed orchards are units for germplasm production that serve as sources of supply that meet the need of seeds in plant production programmes. In seed stands, the trees are not managed so as to increase the genetic diversity of seed, while in seed areas, poorly formed trees are logged and the best quality trees are used to increase genetic gain. Seed orchards are planted forests where seeds or clones of selected trees are used, in order to produce seeds frequently, in abundance, and of a high genetic quality and ease of harvest (Prieto & López, 2006).

Since 1986, Durango State has ranked first in forestry production in Mexico; it has an annual average production of 1,757,638 m^3 (Corral *et al*., 2009). *Pinus durangensis* Mart. is one of the most important trade species in the State because of its physical and mechanical wood properties, wide distribution, as well as the harvested volume (Corral *et al*., 2004), this species is endemic, forestry and ecologically important; for these reasons, it is widely used for reforestation programmes (García & González, 1998). Nearly 12 million plants of *Pinus* are produced annually (Prieto *et al*., 2012). Despite the large number of seeds needed to meet plant production programmes, there are no systematic programmes for collection and management of seed, which means that in many cases the quality and quantity of collected germplasm is unknown.

The main objective of this research was to determine the productive potential, production efficiency and seed quality in seed areas of *P. durangensis* Mart, in La Florida and La Campana and in the Pericos seed stands, located in the municipalities of San Dimas and Pueblo Nuevo in Durango, Mexico.

Materials and Methods

The study sites were one seed stand and two seed areas of *Pinus durangensis* Mart, located in the municipalities of San Dimas and Pueblo Nuevo in Durango state, Mexico, at the Sierra Madre Occidental (Table 1). Ten trees from each site were randomly selected. The minimum separation between trees was 50 m, intended to minimize the kinship between them. Ten cones from each tree were randomly collected and placed in paper bags. In total, 100 cones were collected from each study site. The cones were dried by exposing them to the sunlight. Seeds were obtained and classified after the scales were separated from each cone by boring holes made with an electric drill.

The scales were quantified and classified into two types: 1) fertile scales; those that have the ability to produce seeds and were located in the middle part of the cone, and 2) upper and lower infertile scales; those that could not be pollinated during pollen dispersal and were located at the top and bottom of the cone. Fertile scales were further classified by the size of the mark left by seed during its development: i) ovules aborted during the first year (seed < 2 mm); ii) ovules aborted during the second year (2 mm < seed < 4 mm), and iii) developed seed (seed > 4 mm). The productive potential of the cone was determined by counting fertile scales and multiplying by two, since each scale has the capacity to produce two seeds. In order to determine the percentage of sound and empty seeds, as well as the damaged and malformed seeds by insects, 400 developed seeds were randomly selected from each site and submitted to X-ray tests. They were classified based on the condition of the testa, gametophytic tissue and embryo (Bustamante-García *et al.*, 2012b).

Germination tests were performed according to the standards of the International Seed Testing Association (Anon.,, 1999). 400 seeds were randomly selected from each site, in order to have four replicates containing 100 seeds. The test consisted of disinfecting seeds for five minutes with a solution of 90 mL of distilled water and 10 mL of commercial chlorine. The seeds were washed four times in distilled water, and then placed on absorbent paper. To avoid contamination produced by fungi from the damping-off complex, 1.5 g of 60 Tecto® fungicide per litre of water was applied. Finally, the seeds were placed in a germination chamber at 25–28°C. After sowing, germination was recorded every third day, for 34 days.

The data (in percent) from the X-ray analysis of the seed production efficiency were transformed with the square root of arcsine (Schefler, 1981). Preliminary statistical analysis of data regarding seed productive potential showed neither a normal distribution, nor homogeneity of variance; therefore, a Kruskal-Wallis non-parametric test was performed (Kruskal & Wallis, 1952). On the other hand, seed quality was studied using the parametric Tukey test by means of a completely randomised experimental design. The NPAR1WAY and GLM procedures of SAS/ETS® were used for seed productive potential and seed quality tests, respectively (Anon., 2004). Germination was analyzed using accumulated germination curves with the Kaplan-Meier method (Fox, 1993).

Results and Discussion

The productive potential and developed seeds variables were significantly different (p<0.0001) between sites (Table 2). La Campana and Pericos sites presented the greatest productive potential, with values ranging from 96.1–100.3 seeds per cone, respectively. However, Pericos seed stand achieved a greater proportion of developed seeds per cone (79.7), which means that those seeds completed the reproductive cycle.

The Pericos seed stand had a higher biological capacity to produce seeds compared to both seed areas, despite not having any management practices. The productive potential of the three sites was low when compared to values reported by Solís *et al*., (2007) from seed areas and seed stands of *P. durangensis* (143.4); however, the biological potential was 64.4% lower than was obtained in the present study. The productive potential reported for a seed orchard of *P. monticola* was higher, with 200 seeds per cone, but only 57.5% developed their productive potential (Owens & Fernando, 2007).

The number of aborted ovules in the first year was significantly different (p<0.0001) among sites. The greatest loss occurred in La Campana seed area, with 15.8 aborted ovules (Table 2). On the other hand, in the second year there was no significant difference between sites, with values ranging from 6.7–9.4 aborted seeds per cone. Since the first year showed higher values of aborted ovules than the second year, it is likely to think that this is a more critical time during the seed formation process. The number of aborted ovules in both years was lower than reported by Gómez *et al.,* (2010) for *P. leiophylla* seeds, with 63.7 aborted ovules. Some causes of aborted ovules during seed development could be deficiencies in pollination and damage caused by pests in the early stages of the reproductive process. Meanwhile, in the second year, the abortion of ovules was mainly due to pest or disease problems (Bramlett *et al*., 1977; Owens *et al*., 2008; Gómez *et al*., 2010).

Table 1. Climatological characterization of the study areas.

Study area	Type of area	Geographical localization	Annual mean temperature (ºC)	Annual Mean precipitation (mm)	Elevation (m)
Pericos	Seed stand	23º 44′ 36″ N 105º 30′ 31″ W	11.5	921	2,812
La Campana	Seed area	23º 45′ 36″ N 105º 30′ 50″ W	11.5	921	2,806
La Florida	Seed area	24º 07′ 48″ N 105º 42′ 20″ W	11.1	839	2,678

Source: Comisión Nacional del Agua, 2009

Table 2. Data from Kruskal Wallis tests for seed productive potential.

Variable	Statistics		Seed stand		Seed areas
	χ^2	*p*	Pericos	La Campana	La Florida
Productive potential	24.5	0.000	100.3 ± 1.9a	96.1 ± 3.0a	83.7 ± 2.5b
Developed seed	18.3	0.000	79.7 ± 2.4a	70.9 ± 3.1b	65.2 ± 2.3b
Aborted ovules (First year)	16.4	0.000	13.3 ± 0.9b	15.8 ± 0.8a	11.8 ± 0.6b
Aborted ovules (Second year)	3.4	0.178	7.3 ± 0.5a	9.4 ± 1.0a	6.7 ± 0.5a
Upper infertile scales	16.8	0.000	8.7 ± 0.3b	10.2 ± 0.4a	11.6 ± 1.1a
Lower infertile scales	15.5	0.000	53.1 ± 1.1b	57.4 ± 1.3a	58.2 ± 1.0a

Averages with different letters within rows are statistically different (p<0.0001)
Values represent average ± standard error, *n*=100

Table 3. Percent of seed quality (filled, veined, malformed and damaged by insects) in seed areas and a seed stand of *Pinus durangensis* Mart.

Seed characteristics	Statistics		Seed stand	Seed areas	
	F	*P*	Pericos	La Campana	La Florida
Filled	4.92	0.01	73.4 ± 2.7a	56.9 ± 5.5b	60.4 ± 3.6ab
Veined	5.76	0.001	24.3 ± 2.1b	41.7 ± 5.6a	36.5 ± 3.1ab
Malformed	0.63	0.54	1.1 ± 0.4a	0.8 ± 0.1a	1.9 ± 0.9a
Damaged by insects	0.96	0.39	1.2 ± 0.7a	0.6 ± 0.1a	1.2 ± 0.2a

Averages with different letters within rows are statistically different (p<0.0001)
Values represent average ± standard error, *n*=100

The number of upper and lower infertile scales was significantly different (p<0.0001) between localities. The most upper infertile scales were in La Florida and La Campana, with 11.6 and 10.2 scales per cone, respectively; these sites also showed the fewest developed seeds. The greatest number of lower infertile scales was obtained in La Florida with 58.2; consequently, this was the site that presented the highest number of scales without fertilization.

Regarding the filled and veined seeds, there were highly significant differences (p<0.01) between sites (Table 3). The highest percentage of filled seeds was obtained from the Pericos seed stand with 73.4%. La Campana seed area showed the greatest amount of veined seeds per tree with 41.7%, a value reflecting the low percentage of filled seeds. The high percentage of empty seed was due to damage by insects during the early stages of seed development and poor pollination. The percentage of filled seeds (56.9-73.4%) was lower than observed by Álvarez & Márquez (1994) for *P. cooperi* var. *ornelasi* (80.0%).

The proportion of malformed seeds and seeds damaged by insects was not significantly different between sites (p>0.01). Malformed seed percentage showed low values ranging from 0.8–1.9% of seeds per cone, and so these trees did not appear to be having physiological problems. Seed damaged by insects ranged from 0.6–1.2% of seeds per cone; therefore, the most damage by insects occurred during seed development. Strong *et al.,* (2001) reported that *L. occidentalis* affected seed production in orchard seedlings of *Pinus contorta* var. *latifolia* Engelmann. Solís *et al*., (2007) found that the highest percentage of seeds damaged in seed areas and natural stands of *P. engelmannii* Carr and *P. durangensis* Mart. was by insects (17.4%), mainly by *Megastigmus albifrons*. Cibrián *et al*., (2008) found that *Conophthorus* spp. was the insect group responsible for the major seed loss in the most important pine species in Mexico. In attacks on the cones and seeds of pine trees, the damage can exceed 60.0% of the seed harvest. Bustamante-García *et al*., (2012b) reported that *L. occidentalis* was responsible for a high proportion of empty seeds in *P. engelmannii* during the initial phase of embryo and gametophyte tissue formation, and that the damage continued throughout development to maturation.

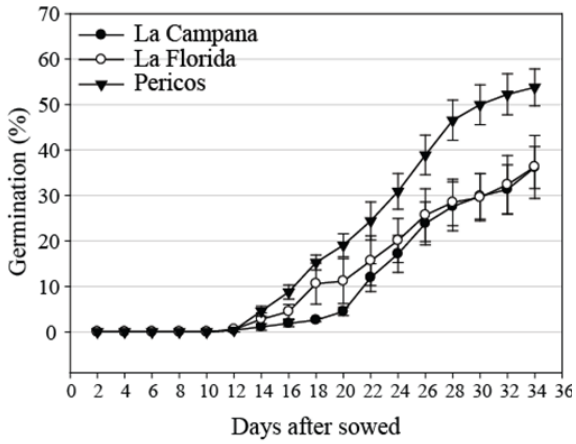

Fig. 1. Accumulated germination curves of *Pinus durangensis* Mart. seeds collected from two seed areas (La Campana and La Florida) and a seed stand (Pericos) from Durango, Mexico. Values represent average ± standard error, *n*=100.

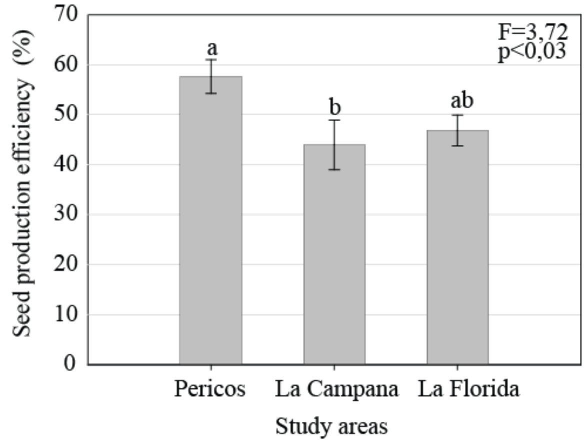

Fig. 2. Seed production efficiency of *Pinus durangensis* Mart. in a seed stand (Pericos) and seed areas (La Campana and La Florida) from Durango, Mexico. Values represent average ± standard error, *n*=100.

Accumulated germination curves analyzed by the Kaplan-Meier method showed that the Pericos seed stand had the highest vigour 26 days after sowing, with an accumulated germination of 38.9% (Fig. 1). According to the germination results (36.2-53-8%), percentages from the three sites were low, indicating a deficient viability and seed vigour. The results for seed production efficiency showed statistically significant differences between sites (p<0.001). The Pericos seed stand had the highest seed production efficiency, with 57.6% (Fig. 2), so it was concluded that good management practices were in place; on the other hand, the seed areas did not exceed values of 46.8%. According to Bramlett *et al*., (1977), seed production efficiency for La Florida and Campana seed areas is deficient. Additionally, low germination values indicate low seed vigor, which is presumably due to insect damage and improper site management.

Bustamante-García *et al*., (2012a) reported that at a seed stand of *Pinus engelmannii* Carr. existed a high value of germination with 98.1% and the large seed production efficiency with 66.9%. Sivacioglu & Ayan (2008) analyzed seed production in a 13 years-old of scots pine (*Pinus sylvestris*) clonal seed orchard, including 30 clones and found a low seed production efficiency (17.9%), this value is quite low in comparison to literature, especially to southern pines in USA. Also, Morales-Velázquez *et al*., (2010), made a cone analysis and found only 1.2 filled seeds per cone or 1.97% of filled seed efficiency; mean reproductive efficiency was low compared to similar reports in the same and in different conifer species. The authors indicated that probably, these results are due to the low pollen production per area, which is associated to the low *Pinus leiophylla* tree density and the presence of endogamic processes. On the other hand, Alba *et al*., (2005) found that the seed production efficiency of *Pinus greggii* ranged from 70.9–87.0%. Analyzing cones and seeds in seed areas and seed stands of *P. engelmannii*, *P. cooperi* and *P. durangensis*, Solís *et al*., (2007) reported the highest seed production efficiency in a *P. engelmannii* seed area (59.5%), and the lowest values in seed areas of *P. durangensis* (21.3% and 36.0%).

From these results, it would appear necessary to continue studying cone and seed production efficiency at the studied sites and other representative areas; this knowledge would indicate whether there is variation between production cycles, so that in a future we could have greater knowledge about this phenomenon. These studies will provide the basis for decision-making in programmes for seed collection, plant production and reforestation.

Conclusions

The Pericos seed stand showed the highest values of productive potential, developed seed, percentage of filled seeds, germination percentage, seed vigor and seed production efficiency. However, the seed vigour was low, so it is not recommended for use in plant production. La Florida and La Campana seed areas showed low values for the examined variables, since the establishment of these areas is based on age and phenotypic qualities of trees. It is necessary to promote better management practices, and thus have more control over seed production in seed stands and seed areas. In addition, it should be considered that low germination values correspond to low seed vigor, reason by which it can be inferred the existence of damage by insects, problem that can be reduced, if management plans are considered.

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