


# Growing *Pinus maximartinezii* Rzedowski in a nursery, in three types of containers: an endangered species endemic to Mexico

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## ABSTRACT

**Objective:** To evaluate the morphological growth of *Pinus maximartinezii* produced in three container sizes at the nursery stage.

**Methodology:** For eight months, plant growth was evaluated in three container sizes: BP-1000 mL, C-200 mL, and C-170 mL. The measured variables were height, diameter, number of whorls, aboveground, root, and total dry biomass, and Dickson quality index.

**Results:** Container size had a significant effect on the morphological variables evaluated ( $p \leq 0.05$ ). The type and size of container influenced the growth of *Pinus maximartinezii* plants. The larger the container volume, the greater the plant growth.

**Conclusions:** The use of larger containers improves the morphological quality of *Pinus maximartinezii* plants in the nursery.

**Keywords:** plant quality, containers, blue pine, stone pine, Dickson quality index.

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## INTRODUCTION

The species *Pinus maximartinezii* Rzedowski, commonly known as blue pine, is endemic to Mexico and is classified as “Endangered” according to the NOM-059-SEMARNAT-2010. It has a restricted distribution in the state of Zacatecas, particularly inhabiting Cerro de Piñones in the Sierra de Morones, municipality of Juchipila, where it occupies 400 ha, with an estimated population of 2,000 to 2,500 trees. In the state of Durango, it grows near the settlement of La Muralla, belonging to the indigenous communities of Santa María de Ocotán and Xoconoxtle (22° 53’



25° N, 104° 13' 31" W, 2,083 m elevation), covering 110 ha with about 900 individuals in scattered patches; it primarily inhabits slopes and ravines with steep gradients (González-Elizondo *et al.*, 2011). Studies on *P. maximartinezii* are scarce: Ledig *et al.* (2001) suggested that *Pinus pinceana* could be a probable derivative from *P. maximartinezii*. Robledo *et al.* (2009) developed a protocol for *in vitro* differentiation of adventitious shoots in cotyledons, and Ojeda-Zacarias *et al.* (2015) reported that this species is amenable to *in vitro* propagation. Moreover, Almaraz-Llamas *et al.* (2019) conducted a morpho-anatomical description of the *Cenococcum geophilum* morphotype associated with *P. maximartinezii* in its natural distribution area for conservation purposes. Natural regeneration of *P. maximartinezii* is limited due to uncontrolled seed collection for human consumption, overgrazing, induced wildfires, and land-use change (López-Mata *et al.*, 2013). Nursery propagation may help conserve the species, but appropriate strategies must be developed for its production in nurseries.

To date, specific protocols to optimize the production of *P. maximartinezii* seedlings remain unknown, unlike for other species of the genus (Escobar-Alonso & Rodríguez-Trejo, 2019). In Mexico, use of polyethylene bags for producing forest species in nurseries has existed for over a century. This traditional system is characterized by the use of polyethylene bags as containers, native soil as substrate, and little or no use of synthetic fertilizers (Aldrete *et al.*, 2024).

Only in 1993 were rigid plastic and polystyrene containers introduced for forest seedling production (Aguilera-Rodríguez *et al.*, 2016), marking a shift from the traditional system to a semi-technified or fully technified one. That shift occurred largely without consideration of the morphological and physiological differences among forest species, the purpose of reforestation, site conditions, or planting methods (Aldrete *et al.*, 2024). Given that container size and type influence plant morphology and physiology especially the root system (Domínguez-Lerena *et al.*, 2006; Vieira *et al.*, 2019) these factors become critical in defining how seedlings should be produced.

Most current containers are rigid, with internal vertical ribs to direct root growth downward, prevent root spiraling, and promote abundant fibrosity and balanced root architecture; they also include drain holes at the bottom to allow root pruning (Haase *et al.*, 2021). In contrast, polyethylene bags have smooth, round walls; when roots reach the bag walls, they coil or concentrate at the bottom, leading to root system deformities (Haase *et al.*, 2021). This problem becomes particularly critical when planting is delayed (Aldrete *et al.*, 2024). Comparative studies of container types and sizes have demonstrated that the volume of the container used in nurseries affects survival and growth in height and diameter across various *Pinus* species (Grossnickle & El-Kassaby, 2016; Madrid-Aispuro *et al.*, 2021). Also, container color and material properties can influence temperature and moisture conditions in the root zone, which impacts plant growth (Sánchez-Aguilar *et al.*, 2016). In this context, the aim of the present study was to evaluate the morphological growth of *Pinus maximartinezii* seedlings produced in three container sizes during the nursery stage. We hypothesize that the larger-volume container will promote superior morphological growth in the seedlings.

## MATERIALS AND METHODS

### Seedling production in the nursery

The experiment was conducted in the “Praxedis Guerrero” forest nursery, municipality of Durango, Durango, Mexico (23° 56' 58.3" N, 104° 34' 07.4" W; elevation 1,890 m). Seeds were collected from *Pinus maximartinezii* trees located near the settlement of La Muralla in the Santa María de Ocotán/Xoconoxtle indigenous community, municipality of El Mezquital, Durango (22° 53' 25" N, 104° 13' 31" W; elevation 2,083 m). Before sowing, as a pre-germinative treatment, seeds were soaked in ambient temperature water for 48 h to enhance germination, and then disinfected with 10% sodium hypochlorite solution to prevent damping-off disease. Sowing was done on 13 January 2017 in seedbeds using a substrate mixture of peat moss (30%), composted bark (30%), perlite (20%), and vermiculite (20%). The nursery period (from 13 January to 5 May 2017) was conducted in a metal-frame greenhouse covered with white polyethylene (720  $\mu\text{m}$ ) and overlaid with a 50% shade cloth; temperature ranged from a minimum average of 6.6 °C to a maximum of 50.1 °C.

Germination occurred on 5 February 2017 (day 23). At 30 days after germination, seedlings were transplanted into the three container types under evaluation. The same substrate mixture as in the seedbed was used, augmented with 3.0 g L<sup>-1</sup> of controlled-release fertilizer Polyblen<sup>®</sup> (12-25-12 N-P-K, 8-9 months release). A mycorrhizal inoculant, Biobravo<sup>®</sup>, containing *Azospirillum brasilense* (5 × 10<sup>8</sup> spores per dose), *Glomus* spp. (80,000 spores per dose), and *Trichoderma* spp. (1 × 10<sup>8</sup> spores per dose), was applied once at 3 mL L<sup>-1</sup> of water using a 425<sup>®</sup> backpack sprayer. Every two weeks, soluble fertilizer Poly-Feed<sup>®</sup> was included in irrigation water. From 5 March to 5 May 2017, an N-P-K formulation 8-52-17 was applied at 3 mL L<sup>-1</sup> of water within the greenhouse conditions described. From 6 May to 6 July 2017, plants were fertilized with 20-9-20 (N-P-K) in the same dose and grown under 50% shade conditions, at an average temperature of 17.5 °C and relative humidity of 38.5%. From 7 July to 7 September 2017, the formulation 22-9-20 (N-P-K) was applied in the same fashion. During this latter period, plants were exposed to outdoor conditions, with an average temperature of 23.0 °C and relative humidity of 41.0%.

### Treatments and experimental design

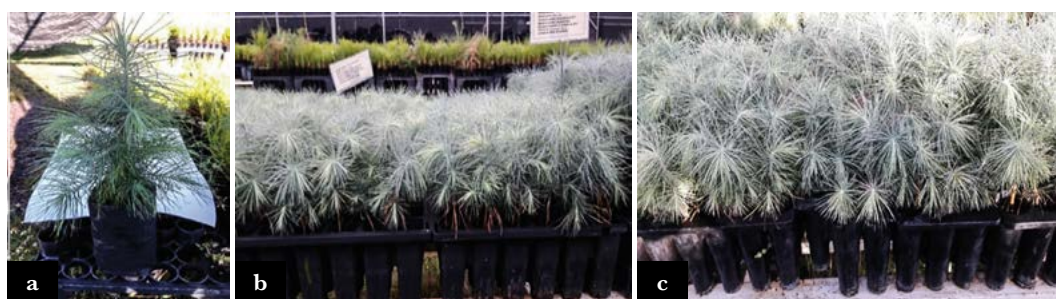
Three container sizes were evaluated (Table 1). The experimental unit was determined by the number of containers or cavities per tray: (1) for the polyethylene bag, being individual, nine plants were used; (2) for the 200 mL container, 60 plants were allocated (10 × 6); and (3) for the 170 mL container, 54 plants were used (6 × 9) (Table 1). Each treatment consisted of four replicates and was arranged in a completely randomized block design.

### Evaluated variables and statistical analysis

Plants were evaluated at eight months of age. From each replicate (tray of 60 cavities, tray of 54 cavities, and nine plants in polyethylene bags), eight plants were sampled (32 plants per treatment). For each plant, the following variables were measured: shoot height (with a PILOT<sup>®</sup> graduated ruler), root collar diameter (with a Truper<sup>®</sup> digital caliper, model CALDI-6MP), number of whorls, and dry shoot biomass (DSB), dry root biomass (DRB),

**Table 1.** Characteristics of the containers evaluated in the nursery production of *P. maximartinezii*.

FEATURES	Polyethylene bag (BP-1000 mL)	Plastic tray (C-200 mL)	Plastic tray (C-170 mL)
Volume (mL)	1000	200	170
Height (cm)	20	13	15
Top diameter	9.6	4.8	5.2
Cavities (No.)	Not applicable	60	54
Shape	Cylindrical	Quadrangular Conical	Conic
Density (plants per m <sup>2</sup> )	100	279	340

**Figure 1.** *P. maximartinezii* produced in three container types in the nursery: a) BP-1000 mL, b) C-200 mL, and c) C-170 mL.

and total dry biomass. Plants were cut at the root collar and both parts were placed in pre-labeled paper bags and dried in a forced-air oven (Felisa<sup>®</sup> model FE-291D) for 72 h at 70 °C. Samples were then weighed on an Ohaus<sup>®</sup> digital scale (model PA1502, precision 0.001 g). Using this data, the Dickson Quality Index (DQI) was calculated (Dickson *et al.*, 1960).

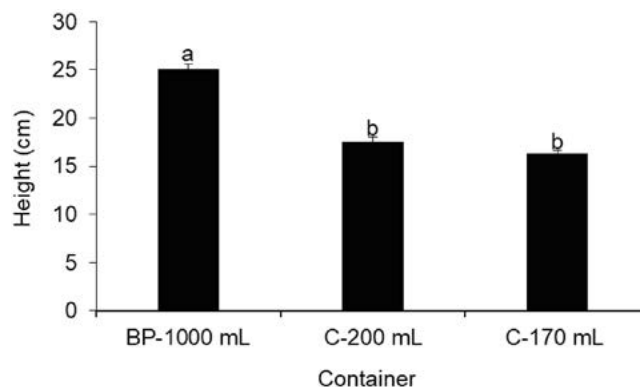
$$DQI = \frac{\text{Total dry weight (g)}}{\frac{\text{Height (cm)}}{\text{Diameter (mm)}} + \frac{\text{Stem shoot part dry weight (g)}}{\text{Root dry weight (g)}}}$$

The effect of treatments (container size) on the evaluated variables was determined through a one-way analysis of variance using the PROC GLM procedure in SAS 9.2<sup>®</sup>. Statistical significance for hypothesis testing was set at an alpha level of 0.05. For variables showing significant effects, Tukey's multiple mean comparison test was applied. In all cases, the assumptions of data normality and homogeneity of variances were verified using the Shapiro-Wilk and Levene tests, respectively (Statistical Analysis System [SAS] Institute, 2009).

## RESULTS AND DISCUSSION

### Morphological variables

Container size had a significant effect on the morphological variables evaluated ( $p \leq 0.05$ ). In terms of height, plants grown in polyethylene bags (BP-1000 mL) stood out, with 8.7 cm more than those grown in the smallest container. Plants grown in C-200 mL

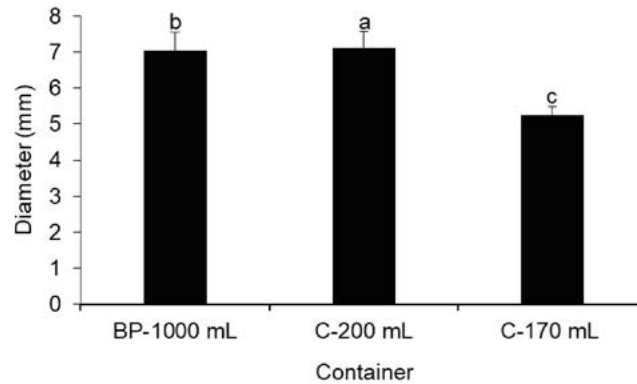


**Figure 2.** Height growth of *P. maximartinezii* in three container sizes. Means with different letters in each bar are statistically different between treatments (Tukey,  $p \leq 0.05$ ).

and C-170 mL containers showed similar values (Figure 2). However, all three container types produced plants with heights exceeding 15 cm, which meets the recommended range established by the Mexican Standard for the Certification of Forest Nursery Operations NMX-AA-170-SCFI-2016 for *Pinus maximartinezii* plants (15 to 20 cm) (Secretaría de Economía, 2016).

Several studies have been conducted regarding the type and size of container in the nursery production of *Pinus* species. The results have varied depending on the species. For example, Bonilla *et al.* (2014) evaluated three container sizes (123, 205, and 305 mL) on the plant quality of *P. tropicalis* Morelet and found that the 205 mL container was the most favorable for plant height. Madrid-Aispuro *et al.* (2021) assessed different container types and volumes (ranging from 160 to 380 mL) for *Pinus cembroides*, finding that the 170 mL polystyrene tray produced tallest plants. Although the best results in these studies were not always associated with the largest containers, most research has identified a positive relationship between container volume and plant quality. This correlation has been demonstrated in several pine species, such as *P. durangensis* Mart., *Pinus cooperi* Blanco, and *Pinus engelmannii* Carr., which showed greater height in larger-volume polystyrene tray containers (Prieto *et al.*, 2006; Prieto *et al.*, 2007a; Prieto *et al.*, 2007b). Root collar diameter also showed significant differences among the evaluated containers ( $p \leq 0.05$ ). The difference between the highest and lowest values was 2.0 mm, with the C-200 mL container promoting the greatest diameter growth, while the C-170 mL container showed the lowest value (Figure 3).

Contrary to expectations, the largest container (BP-1000 mL) did not produce the highest stem diameter; the difference with the C-200 mL container was only 2.0 mm. In this case, the polyethylene bag with greater depth, wider top diameter, and lower plant density per unit area produced individuals with smaller collar diameters. This response could also be related to the evaluation period (eight months), as plants in polyethylene bags can continue growing without negative effects for a longer period (up to two years), unlike other containers, where the optimal growth window typically does not exceed one year. Several authors have reported favorable effects of container size on diameter growth in *Pinus* species. For instance, Bonilla *et al.* (2014) found that in *P. tropicalis* Morelet, the

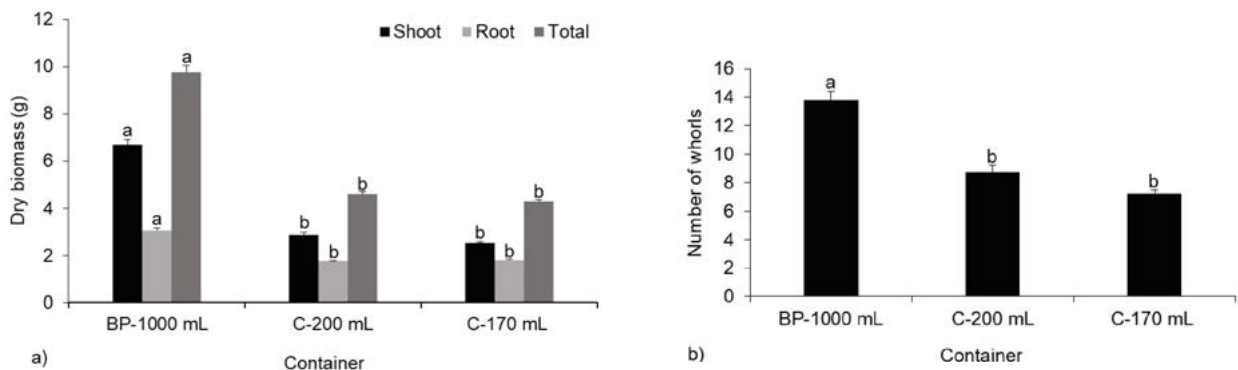


**Figure 3.** Root collar diameter growth of *P. maximartinezii* in three container sizes at the nursery stage. Bars with different letters indicate statistically significant differences between treatments (Tukey,  $p \leq 0.05$ ).

205 mL container led to the highest diameter growth among three sizes (123, 205, and 305 mL). Similarly, González *et al.* (2017) observed that in *Pinus patula* Schl. *et* Cham., the largest diameters were obtained in plants grown in the largest container (165 mL). Madrid-Aispuro *et al.* (2021) also reported that in *Pinus cembroides* Zucc., the greatest field diameter increases corresponded to plants grown in 380 mL tubes. These findings suggest that species-specific evaluations are essential, as each species may respond differently to container volume.

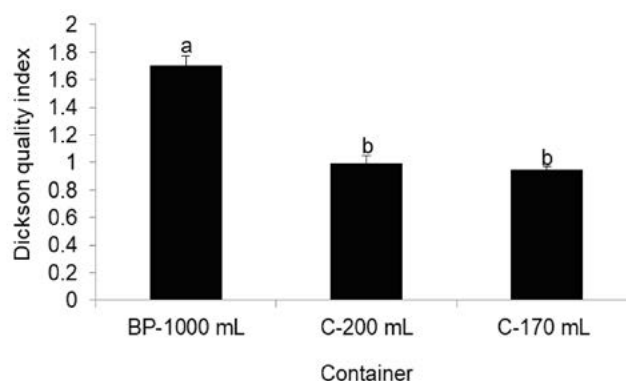
Significant statistical differences were found for shoot, root, and total dry biomass, as well as for the number of whorls ( $p \leq 0.05$ ). Plants grown in polyethylene bags (BP-1000 mL) showed the highest values with 6.7 g, 3.0 g, and 9.8 g, respectively, along with the highest number of whorls (14). Containers C-200 mL and C-170 mL yielded similar values for both biomass and whorls (Figure 4).

Plants grown in polyethylene bags produced more than 50% higher aerial, root, and total biomass compared to those grown in C-200 mL and C-170 mL containers. Jaeyeob *et al.* (2010) evaluated three container volumes (250, 350, and 500 mL) in *Pinus densiflora* and *Pinus thunbergii*, and found that dry biomass in both species was proportional to container volume, with no significant differences between the two smaller volumes. Container size



**Figure 4.** Shoot, root, and total dry biomass (a) and number of whorls (b) in *P. maximartinezii* produced in three container types and sizes in the nursery. Means with different letters within each variable are statistically different among treatments (Tukey,  $p \leq 0.05$ ).

and shape regularly influence root growth, which in turn affects plant development several months after transplanting, impacting survival and plant growth (Mariotti *et al.*, 2015; Montagnoli *et al.*, 2021; Aldrete *et al.*, 2024). In this study, plants produced in polyethylene bags showed no root coiling by eight months of age, a point at which they were ready for field planting. Domínguez-Lerena *et al.* (2006) note that container volume influences plant development because physical restriction of the root system limits both water and nutrient availability. The Dickson Quality Index showed significant differences ( $p \leq 0.05$ ) among container sizes. The highest Dickson index was obtained for plants grown in polyethylene bags (BP-1000 mL), being 50% higher than in C-170 mL; meanwhile, the C-200 mL and C-170 mL containers produced similar values (Figure 5). This index is recognized as one of the best indicators of plant quality, as it integrates multiple growth aspects (Binotto *et al.*, 2010). Its use facilitates the selection of individuals with a higher probability of success in field planting, thereby contributing to improved survival (Tsakaldimi *et al.*, 2013).



**Figure 5.** Dickson Quality Index in *P. maximartinezii* grown in three container types and sizes in the nursery. Means with different letters above each bar are statistically different among treatments (Tukey,  $p \leq 0.05$ ).

Various studies have reported that plant quality parameters increase with container volume (Landis *et al.*, 1990; Kolevska *et al.*, 2020). This work supports the idea that container volume is one of the most important factors in seedling production, especially when the primary objective of reforestation is species conservation, as is the case of *Pinus maximartinezii*. In *Pinus nigra*, *Pinus sylvestris*, and *Pinus pinaster*, the Dickson quality index has been a good predictor of survival (Kolevska *et al.*, 2020). In this sense, Grossnickle and MacDonald (2018) note that reforestation using highquality seedlings increases the chances of successful establishment and growth; however, one must also consider other factors such as the reforestation purpose, site conditions, and planting technique (Tsakaldimi *et al.*, 2013).

## CONCLUSIONS

Using larger containers in the production of *Pinus maximartinezii* seedlings significantly affects their morphological characteristics and, consequently, their quality. This finding underscores the importance of properly selecting propagation systems to improve the probability of field success. Because this is an endemic species with a limited distribution,

optimizing its nursery management is a key tool for its conservation through reforestation programs in its natural habitat.

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