Article

Plant quality and survival in *Pinus montezumae* Lamb. reforestation

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Abstract:

Plant quality of *Pinus montezumae* was evaluated in a nursery, laboratory and plantation experiment. The trees were produced in a forest nursery in *Tetela del Volcán, Morelos* state. Plant quality indicators were measured in a sample. The plantation was made in the foothills of the ***Popocatépetl*** volcano. Plots were established at 3 260 masl and 3 170 masl of the reforested ridges, on northern and southern exposures, with three replicates for each factor combination, with a total of 12. Each plot had a planting frame of 7 × 7, to evaluate the central part (25 specimens) and avoid the border effect. Planting density was 1 200 ha^{-1}. At 22 months after established, height, diameter, apparent state and their condition of live or dead plant were registered. An analysis of variance was applied to the response variables measured in the field. The results indicate differences in survival by exposure (P = 0.0222), with the north (88.6 %) surpassing the south (83.3 %). The quality indicators obtained are predictors of survival. The mean and minimum values (maximum in the case of the dry aerial weight / root dry weight ratio and the slenderness coefficient) were 13.6 and 5 mm for the diameter; 3.0 and 5.2 for the dry aerial weight / root dry weight ratio; 1.2 and 3.9 for the coefficient of slenderness; 4.7 and 1.5 for the Dickson index and 55 and 29 for new roots in the root potential growth test

Key words: Plant quality, exposure, *Pinus montezumae* Lamb., plantation, restoration, *Tetela del Volcán*.

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Introduction

Forests play a key role in climate regulation on the planet and provide goods and services directly or indirectly. Between 2005 and 2010 its area has been gradually reduced by 155 000 ha per year, due to illicit logging, land use change for agriculture and livestock, increased urban sprawl and poor use of fire (Inegi, 2013). By 2014, the *Comisión Nacional Forestal* (National Forestry Commission) recorded 5 332 fires over 155 533.52 ha throughout the Mexican republic, most of them caused by agricultural fires and several due to changes in land use. The latter is the major factor of deforestation, because it generates short-term economic income. Faced with such losses, reforestation and the restoration of ecosystems have increased during the last years in Mexico (Conafor, 2014). Based on the information provided by Inegi (2013), in 2011 2 268.38 km^2 were reforested in the country; unfortunately, the survival of these plantations is low.

Con el propósito de abastecer de planta a dichos programas, existen cientos de viveros forestales en todo el país, y solo una parte de ellos opera con tecnología avanzada, pero se sigue experimentando en la producción, lo que obedece, en parte, a que el número de especies involucradas va en aumento. Con frecuencia no se cuenta con información técnica sobre cómo producir las especies que se trabaja por primera vez.

In areas where reforestation is carried out, survival values are contrasting and are associated with drought, frost, overgrazing or other environmental factors and the inappropriate use of fire, among other reasons; to these the quality of plant, whose contribution is decisive, should be added.

A quality plant is the one that has the attributes to survive and grow at the plantation site (Duryea, 1985a). The subject has decades of research in the United States, and among the outstanding works on plant quality are those of Duryea

(1985b) and Landis *et al.* (2010). In Mexico one of the pioneering contributions is that of Prieto *et al. (*1999), but it is not until the present century that this aspect began to receive more attention in the country.

Rodríguez (2007) reports that the components and operations that can affect plant quality are the size of the bag or container; density; transplant; type of substrate; watering; fertilization; mycorrhizae; temperature; light; removals and root pruning for bag production; pest and disease care; the correct hardening phase; loading, transportation and unloading, and poor concentration, placing and planting.

According to the same author, plant quality control must be visualized in two ways: first, to obtain certain morphological and physiological standards, which denote its quality; and the second, which is related to the specification of those standards that are statistically assessed in the field, which in Mexico have been scarcely evaluated.

The plant quality of *Pinus montezumae* Lamb. has already been studied by Bautista *et al.* (2005) and Hernández *et al.* (2014). However, they do not cover the great diversity of nursery practices, such as fertilization, substrates, containers, among other factors, as well as the varied planting conditions in the field, so there is still ample room for research on the subject and the species. Based on the above, the objective of the present study was to evaluate the effect of plant quality of *P. montezumae* produced in nursery and planted at two different altitudes and exposures.

Materials and Methods

Study area

"Tetela del Volcán" forest nursery depends of the *Secretaría de Desarrollo Sustentable (SDS) (*Department of Sustainable Development) of the *Gobierno del Estado de Morelos* (Morelos State Government) and is located at the municipality of *Tetela del Volcán*, between the geographic coordinates 18°52’27.25” N and 98°42’54.27” W (Figure 1).

Figure 1. Location of *Tetela del Volcán* municipality, *Morelos* State.

There are four kinds of climate in the municipality, according to altitude. The area where the actual study took place is located at 3 200 masl, has a sub-humid semi-cold climate with summer rains, average annual temperature from 16 to 17 °C and 1 073.2 mm average annual precipitation (Inegi, 2009).

Plant production

The plant used for the present work was cultivated in trays of 49 cavities with 150 ml capacity per container. The substrate was a mixture of moss peat (42 %), vermiculite (31 %), agrolite (27 %) and 4 kg m-3 of Osmocote™ 15-9-12 granular fertilizer. At the establishment stage, Initial Ultrasol™ fertilizer 15-30-15, 112 ppm N, 99 ppm P and 93 ppm K was applied. In the growth stage, Ultrasol™ 20-10-20 fertilizer was applied, with 144 ppm N, 32 ppm P and 120 ppm K. For the hardening step, the Peters fertilizer finer 4-25-35, with 26 ppm N, 70 ppm P and 186 ppm K was applied. Mycorrhizal inoculum (Glumix™) was applied to the sowing and to seedlings 20 days later, with 15 g per cavity in each application. As seedlings were not planted, they remained 22 months in the nursery.

Plant evaluation at the nursery

The evaluated plants were randomly selected from the nursery platforms. According to Rojas (2002), the suggested sampling intensity is 0.5 % for populations greater than 50 000 plants. However, he recommends lowering this intensity when it comes to technically-grown nurseries with homogeneous production. In this work a sample of 0.33 % was obtained, and thus 200 plants of the 60 000 that were produced were selected. The height (cm), the diameter of the neck (mm) were measured; the percentage of mycorrhization was estimated, assigning a scale value of 0 to 100, where zero equals the apparent absence of mycorrhizae in the root ball and 100 to be completely filled with mycorrhizae.

Aboveground and radical architecture was also evaluated by observation. A value of 3 was given if there was no damage, 2 if 30 % of the seedlings had any, and 1 if more than 50 % of the plant had. The plants were sent to the reforestation site.

Plant evaluation in the laboratory

In this evaluation a destructible random sample of 0.16 % of the total plants produced was handled. 96 seedlings were taken to the *Laboratorio de Semillas Forestales* (Forest Seeds Laboratory) of the *División de Ciencias Forestales* (Forestry Division) of the *Universidad Autónoma Chapingo* (Chapingo Autonomous University) to measure them.

The same measurement procedure was performed as in the nursery. In addition, the root mass was removed to clean the root, measure its length (cm) and count the secondary roots > 1 cm.

To continue with the evaluation, the needles were separated from the stem, the stem from the root, and each part was weighed separately. They were then pocketed and labeled. The bags were placed inside the drying kilns of the laboratory, at a constant temperature of 75 °C. After 5 to 7 days, weight became constant. Each of the samples was then weighed to obtain total dry weight, as well as that from foliage, stem and root per plant with an Ohaus Navigator scale.

Plant quality indicators

Most of these indicators, and their models, were taken from Duryea (1985b) and Landis *et al.* (2010). The following attributes were considered: plant height, plant neck diameter, percentage of mycorrhizae, shoot and root architecture. In addition, the following indicators of the destructible sample were obtained: main root length and number of secondary roots > 1 cm

To determine the relation between aboveground and root dry weight (A/S), the following model was used:

$$A/S = PSA/PSS$$

Where:

PSA= Dry weight of the aboveground part

PSS = Dry weight of the root

The relative weight of the aboveground part (PR_A) was calculated as follows:

$$PR_A = PSA/PST$$

Where:

PSA= Dry weight of the aboveground part

PST = Total dry weight

The relative weight or of the root or the underground part (PR_R) was determined with the following model:

$$PR_R = PSS/PST$$

Where:

PSS = Dry weight of the root

PST = Total dry weight

The Slenderness Coefficient (CE) was calculated with the following model:

$$CE = (A/D)$$

Where:

A = Height of the plant

D = Diameter of the plant

For Dickson's Index (ID) the following model was used:

$$ID = PST / ((PSA/PSS) + (A/D))$$

Where:

PST = Total dry weight

PSA = Dry weight of the aboveground part

PSS = Dry weight of the root

A/D = Slenderness coefficient

With the data that were obtained in this phase, a Pearson's Correlation was made, by means of the SAS program (SAS Institute, 2012). In addition to evaluating the former indicators, also the following tests for plant quality were made.

Potential root growth test. Four plastic pots of 4 l each were used for this test, which were filled with a mixture of substrate made up by 1/3 agrolite, 1/3 vermiculite and 1/3 peat moss. Afterwards, three seedlings were put into each one. The pots were left inside the greenhouse, where they were watered every two days (at a temperature of 23-28 °C, with a relative humidity of 60-75 %). After that month, they were taken out of the container, with the aim to count the roots larger >1 cm. Such new roots were identified as they were white and turgid (Landis *et al*., 2010).

Water stress test. The Scholander chamber (model 1000 PMS Instrument) was used for this test. Ten plants were used, from which was taken as a simple the high part of their stem with a crosscutting procedure. The sample was inserted in the tap of the chamber, which closes another in which the sample is placed, and to which a N gas pressure was applied. In this way the necessary pressure is made to start the extraction of water from the sample (Landis *et al*., 2010). The first assessment was made to five plants after watering and to the other five, seven days later they were measured, without any more watering during that period, which led to water stress. From the night before they were measured, all the plants were kept in a fresh dark room. For the statistical analysis of this test, a Student's t test was performed in order to compare the means of the plants under the two applied treatments.

Reforestation assessment

The plantation was established in August 2013 and its evaluation was carried out in June 2015, in a period of 22 months. The height, diameter, and vitality condition of each of them (living or dead) were recorded.

Plots were located in the upper part (3 260 masl) and in the low one (3 170 masl) of a hill, in forest soils, of volcanic origin, in north and south exposure, three in each combination of altitude and exposure, *i. e.* 12 plots in total. It was a 2 × 2 factorial experiment. Each plot consisted of a plantation frame of 7 × 7 plants, to evaluate only the central part, 5 × 5 (= 25 plants) to avoid the possible border effect. Planting density was 1 200 ha^{-1}. Therefore, 12 plots with 25 plants each (300 plants in total) were measured; the data were subjected to an analysis of variance using the SAS program (SAS, 2012).

Results and Discussion

Results indicate that the mean heights and diameters for *P. montezumae* are within the quality ranges reported for other pines, such as *P. devoniana* Lindley (Reyes *et al*., 2014, Sáenz *et al.,* 2014). This species is cespitose (Perry, 1991), but not in all its populations, as happens with *P. hartwegii* Lindl. (Rodríguez, 2015). The high variability of heights of the sample suggests that the plant produced at the nursery had different levels of intensity in the manifestation of the mentioned feature. It should also be considered that the plant was in there for several months longer than is usual for cespitose species (Reyes *et al*., 2014) (Table 1).

Table 1. Summary of data of the variables evaluated at the nursery.

Variable	Maximum	Minimum	Average
Height (cm)	37	2	17.8
Diameter (mm)	24	6	15.1
Mycorrhization (%)	100	0	10.3

In regard to the aboveground and root architecture, 77.5 % of the plants have good quality, 17 % medium and 5.5 % bad. This, together with the mycorrhization levels and the high survival formerly detailed, show that this vegetal material is acceptable.

Plant evaluation in the laboratory

Although it is a plant that spent almost two years in the nursery, it exceeds the basal diameter standards established for the species, from 6 to 11.5 mm (Bautista *et al.,* 2005; Hernández *et al*., 2014) or for *P. devoniana*, with up to 13.5 mm (Sáenz *et al.,* 2014; Bernaola *et al.,* 2015); both species remained in the nursery for a year in similar containers, ranging from 160 to 220 cm^3.

The calculated PSA / PSS ratio was rather high, equal to 3. However, such a value was lower than those obtained in other investigations, such as the interval reported by Aguilera *et al.* (2016), from 3.1 to 4.6 in different fertilization treatments, although they used larger containers, 10 cm^3 than in this work. It should be noted that, of the studies cited in this section, only that of Bautista *et al.* (2005) considers establishment of plantation in the field, like the actual work.

Another indicator, the Dickson index, yielded high values, although the recommendation of Sáenz *et al*. (2014) for another cespitose species, *P. devoniana*, is ≥ 1.2. The laboratory data are in Table 2.

Pearson's Correlations of the data from the 11 variables evaluated in the laboratory (Table 3) only indicate those that were significant among the assessed variables with $p < 0.05$ and with the correlation coefficient ≥ 0.4. They emphasize simple variables of measure, like height and diameter, but in particular total dry weight.

Potential root growth test

A vigorous plant response was observed for the production of new roots (mean of 55.3, maximum, 86 and minimum, 29), which is a good indicator of plant quality, since the seedling will have greater facility to acclimatize to the site and therefore their development will be better and their survival rate increased (Ritchie and Landis, 2003).

Table 2. Basic statistics of the assessed variables at the laboratory.

Variable	Mean	Standard deviation	Minimum	Maximum
Diám (mm)	13.6	3.5	5	25
Alt (cm)	14.9	8.6	2	40
Mico (%)	9.4	18.4	0	100
Raíz (cm)	11.6	5.5	4	42
Raíces	14.6	3.9	7	31
Arq	2.6	0.7	1	3

PSA (g)	4.2	1.9	0.7	9.6
PST (g)	3.4	2.0	0.5	10.3
PSR (g)	2.6	1.2	0.4	5.9
PSA/PSS	3.0	0.8	1.0	5.2
PSA/PST	0.7	0.1	0.5	0.8
PSS/PST	0.3	0.1	0.2	0.5
CE (cm/mm)	1.2	0.7	0.1	3.9
ID	4.7	1.8	1.1	9.7

Diám = Diameter of the neck; *Alt* = Height; *Mico* = Percent of mycorrhization of the root mass; *Raíz* = Length of the main root; *Raíces* = Number of roots larger than 1 cm; *Arq* = Architecture of the aboveground and root parts; PSA/PSS = Relation between the dry weight of the aboveground part and dry weight of the root; PSA/PST = Relative weight of the aboveground part; PSS/PST = Relative weight of the root; CE = Slenderness coefficient; ID = Dickson's Index.

It is considered that for the pine species studied, an average of 55 new roots (minimum 29) generated in the potential root growth test, implies the average survival obtained in reforestation areas, with 88.6 % for northern exposures and 83.3 % for the southern, under the experimental conditions described. It is worth mentioning that in other studies, a smaller number of new roots are mentioned in similar periods, such as Simpson and Vyse (1995), who with more than 10 new roots determined a survival in the field greater than 70 % in *Picea glauca* (Moench.) Voss and *Pinus contorta* Douglas.

Table 3. Pearson's Correlation.

Variables		**R^2**	**P**
Diám	PSA	0.53097	< 0.0001
Alt	PSA	0.4043	< 0.0001
Mico	PSA	0.46079	< 0.0001
Raíz	*Raíces*	0.43885	< 0.0001
PST	*Diám*	0.43251	< 0.0001
PST	*Alt*	0.58125	< 0.0001
PST	*Mico*	0.46416	< 0.0001
PST	PSA	0.85309	< 0.0001
PSR	*Diám*	0.56928	< 0.0001
PSR	*Mico*	0.41354	< 0.0001
PSR	PSA	0.78232	< 0.0001
CE	*Alt*	0.87445	< 0.0001
ID	*Alt*	0.64378	< 0.0001
ID	PSA	0.62388	< 0.0001

Diám = Diameter of the neck; *Alt* = Height; *Mico* = Percent of mycorrhization of the root mass; *Raíz* = Length of the main root; *Raíces* = Number of roots > 1 cm; PSA = Dry weight of the aboveground part; PST = Total dry weight; PSR = Relative weight or of the root or the underground part; CE = Slenderness coefficient; ID = Dickson's Index.

In favorable environments and with species that are good producers of new roots, the CPR test provides its best results (Ritchie and Landis, 2003). The number of new roots referred in the previous paragraph for the three species compared, suggests that *P. montezumae* has that ability. The fact that the plantation environment was not limiting, which shows the good survival obtained, indicates that, for this species and the selected locality, CPR was satisfactory. This procedure must be carried out under conditions similar to the plantation site to have a result that is closer to reality (Folk and Grossnickle, 1997), as it was done in the present work.

Water stress test

Student's t test showed differences between plant readings with and without water stress ($p = 0.029$). The plants irrigated the day before this test, had an average of 0.71 MPa, while those that remained without irrigation for a week reached 1.36 MPa. According to Landis *et al.* (1989), the first figure is within the recommended range for the hardening period, when growing forest species in containers. The second one overcomes the stress that must be submitted to the plants in the nursery, even during the hardening stage, since it should not exceed 1 MPa ever, as this affects growth and increases the probability of damage and mortality. On the other hand, this test shows that every day that passes without watering, the plant increases the tension by 0.19 MPa.

Reforestation assessment

According to the comparison of nursery and field evaluation, during the planting time, the seedlings had a growth of the order of 1 cm in height and 1.5 to 2.5 mm in diameter, under the different conditions of the experiment (Table 4). Although survival is high, the low growth in height is due to the fact that it is a cespitose species (Perry, 1991).

No significant differences were detected by any individual factor, nor by their interaction, neither for height nor for diameter. In the case of survival, there was an effect of exposure (P = 0.0222), with the north (88.7%) surpassing the south (83.3%) (Table 5). Data from the *Tetela del Volcán* weather station indicate that the years 2013 and 2014 were wet, with 1 293.5 and 1 179.6 mm, respectively. So abundant precipitation could contribute to the fact that the moisture difference between exposures was smaller, with a small difference in survival and non-significant differences in diameter. Survival on the southern slope was similar to that reported by Bautista *et al.* (2005) (83.8%) for the same species, at slightly higher (3 368 masl) and flat (2 % slope) sites in central Mexico. This plant was established with diameters at the base > 6 mm, but survival on the northern slope exceeded this last figure, possibly because it is wetter than flat terrain.

Table 4. Reforestation assessment, 22 months after established.

Exposure	**Altitud (masl)**	**Average height (cm)**	**Average diameter (mm)**	**Average survival (%)**
North	3 260 (High)	19.07	17.63	89.3
North	3 170 (Low)	18.65	16.65	88
South	3 260 (High)	18.94	16.67	80
South	3 170 (Low)	18.83	17.19	86.6

Table 5. Factor significance for height, diameter and survival.

Variable	Factor or Interaction	P
Height	Exposure	0.958
	Altitude	0.5614
	Exposure * Altitude	0.7301
Diameter	Exposure	0.7416
	Altitude	0.7097
	Exposición * Altitud	0.2459
Survival	Exposure	0.0222
	Altitude	0.195
	Exposure * Altitude	0.0667

Conclusions

A quality *P. montezumae* plant was obtained with high survival in the field (> 83 %), 22 months after it was planted. The seedlings reached satisfactory development, but it is considered that the production can be achieved in less time and that the fertilization schemes can be refined.

The quality parameters obtained in the present study can be considered as predictors of survival of at least 83 % in the study conditions, including two years without moisture limitations. Despite the high humidity during the planting period of the study, the northern exposure recorded higher survival, which is related to the even greater humidity that typifies such exposure in the northern hemisphere. No effect of altitude was confirmed, possibly because the difference between the plots was very low (90 m).

It is possible that such indicators are valid also for dry years, although maybe with less survival, because the plant is robust and with high diameters and root amount. However, it would be advisable to increase the supply of mycorrhizal fungi, since under the conditions that the nursery produces today, the plant exhibits its low content.

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Conflict of interests

The authors declare no conflict of interests.

Contribution by autor

Fernando Robles Villanueva: research design, field and laboratory work, data analysis, writing and editing of the manuscript. Dante Arturo Rodríguez Trejo: research design, research direction, field and laboratory work, data analysis, writing and editing of the manuscript. Antonio Villanueva Morales: research design, research direction, data analysis, writing and editing of the manuscript.