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Research article

Fertilización, calidad de planta y supervivencia en campo de Pinus spp. en Ixtlán de Juárez, Oaxaca

Fertilization, plant quality and field survival of Pinus spp. in Ixtlán de Juárez, state of Oaxaca

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Abstract

The *Pinus patula* and *Pinus oaxacana* seedling quality was evaluated under three fertilization treatments. The production cycle spanned along 9 months in the *Ixtlán de Juárez* forest nursery, state of *Oaxaca*, Mexico. The assessed variables were: caliper, height, tap root length, shoot, root and total dry weight, shoot/root ratio, slenderness coefficient, lignification index and Dickson index, as well as the root growth potential. Field survival 12 months after planting in localities of the same community was analyzed. The seedlings of both species reached a caliper ≥3.5 mm and heights from 28 to 42 cm. The mean values were: 3.5 g (total dry weight), 4.4 (shoot/root ratio), 8.1 (slenderness coefficient), 29.7 % (lignification index), 0.25 (Dickson index). After the application of fertilization treatments high and the traditional for the forest nursery, in both of the species were obtained mean and high quality seedlings. 12 months after planting, the mean survival was equal to 47 %. There were statistical significant differences for aspect (p=0.0266) and fertilization (p≤0.0001), but not for species (p=0.7604). The variables more closely related to the mortality risk in the plantation site were aspect, fertilization, caliper and root growth potential.

Key words: Plant quality, *Pinus patula* Schltdl. & Cham., *Pinus oaxacana* Mirov, seedling production, reforestation, forest nursery.

Resumen

Se evaluó la calidad de planta producida con tres tratamientos de fertilización en *Pinus patula* y *Pinus oaxacana*. Las plantas se produjeron durante nueve meses en el vivero forestal de Ixtlán de Juárez, Oaxaca, México. Las variables medidas fueron: diámetro al cuello de la raíz, altura, longitud de raíz principal, peso seco aéreo y de raíz, peso seco total, relación peso seco aéreo/peso seco de raíz, se calcularon el coeficiente de esbeltez, los índices de Lignificación y de *Dickson*, además del crecimiento potencial de raíz (*CPR*). Se analizó la supervivencia en campo a 12 meses de haberse plantado en áreas de la misma comunidad. En ambas especies se obtuvo planta con diámetro ≥3.5 mm y alturas de 28 a 42 cm. Los valores medios de las plantas fueron: 3.5 g (peso seco total), 4.4 (relación peso seco aéreo/peso seco de raíz), 8.1 (coeficiente de esbeltez), 29.7 % (Índice de Lignificación), 0.25 (Índice de *Dickson*). Al aplicar la fertilización alta y la tradicional del vivero en las dos especies, la planta fue de calidad media y alta. A 12 meses de la plantación, la supervivencia promedio fue de 47 %. Se determinaron diferencias significativas para exposición (p=0.0266), esquema de fertilización aplicado (p≤0.0001), pero no entre especies (p=0.7604). Las variables que más se relacionaron con el riesgo de mortalidad en el sitio de plantación fueron: exposición, fertilización, diámetro y *CPR*.

Palabras clave: Calidad de planta, *Pinus patula* Schltdl. & Cham., *Pinus oaxacana* Mirov, producción de planta, reforestación, viveros forestales.

Introduction

Recent studies on a global scale conclude that in the last 50 years, humans have modified the planet's ecosystems more quickly and extensively than in any other period in history (Aleixandre-Benavent *et al.,* 2018). Deforestation is a problem for developing countries, since it causes biodiversity loss and potentiates the effects of climate change (Hein *et al.*, 2018).

Mexico is not exempt from these trends (Barrera *et al*., 2018). Reforestation programs in Mexico are a permanent strategy to recover, maintain and increase forest areas and reduce the degradation of forest lands (Flores *et al.*, 2021).

The success or failure of these reforestation programs is linked to climate (Barrera *et al.*, 2018), although the yield of the plants in the field is affected by their quality and by the prevailing conditions of the reforestation site (Grossnickle and MacDonald, 2018).

There are hundreds of forest nurseries in the country that supply plants to these reforestation programs, but only some use advanced technology (Robles *et al.*, 2017). These nurseries seek to generate quality plants, base their production on containers with a correct plant density, appropriate substrate, adequate irrigation and fertilization schemes, among others, which are the components and operations that most affect plant quality (Rodríguez-Trejo, 2008).

The *Pinus* genus is the most used one for reforestation in Mexico from its ecological, economic and social importance (Flores *et al.*, 2021). The species of this group are a source of wood, firewood, pulp, resins and other products (Farjon and Filer, 2013). In nurseries, it is of interest to provide optimal fertilization, since excesses, even if they do not reach toxic levels, increase production costs. In *Ixtlán de Juárez*, *Pinus patula* Schltdl. & Cham. and *Pinus oaxacana* Mirov are dominant in the forests that are managed for their use. In this context, the objectives of this study were: to evaluate the quality of the nursery plant produced with three fertilization schemes for these species, and its survival in the field after 12 months of planting in the reforestation areas of the same community, and calculate the risk of mortality of the plants based on their morphological variables and the conditions of the site.

Materials and Methods

Study area and plant production

The study was carried out in the technified forest nursery of the *Ixtlán de Juárez* community in the *Sierra Norte* of *Oaxaca* (17°20' N and 96°29' W, at 2 030 masl). The annual average temperature is 18.3 °C and the annual mean precipitation is 759.3 mm (Villegas-Jiménez *et al.*, 2016). Species scheduled for reforestation in August-September 2021 were used: *Pinus patula* and *P. oaxacana*. Seeds were collected from “plus trees” for timber production in the communal forests of *Ixtlán* *de Juárez.* Sowing was made in polyethylene containers (24 cavities, 143 mL each). As a substrate, a mixture of peat moss, agrolite and vermiculite (40:20:40) was prepared. Slow release granular fertilizer Multicote Agri® (Haifa) formulation 18-6-12 of N-P-K (2.5 kg-m^3 of substrate) was applied.

Nutrition was based on the application of soluble Foresta® fertilizer (Foresta) with formulations according to the cultivation stage: establishment (formulation 9-45-15, N-P-K), growth (20-10-20) and hardening (4-25-35). This fertilization was applied in two doses: low dose (100 ppm P, 64.2 ppm K and 46.4 ppm N [establishment], 100 ppm N, 83 ppm K and 21.5 ppm P [growth], and 125 ppm K, 46.2 ppm P and 17.2 ppm N [hardening]) and high dose (120 ppm P, 77 ppm K and 56 ppm N [establishment], 120 ppm N, 99.6 ppm K and 25.8 ppm P [growth], and 150 ppm K, 55.9 ppm P and 20.8 ppm N [hardening]). In addition, a third treatment (called "traditional nursery dose") was included, which consisted only of the addition of inorganic solid mineral soluble fertilizer ELIXIR SUPREME® (11-12-18), with application of 0.6 g L^{-1} every four weeks to the base of the stem of the plant from the 5th week.

Plant quality analysis

After nine months of growth, seven seedlings from each of the six treatments (3 doses × 2 species) were randomly taken to measure: stem caliper (*Dc*, mm, with Truper® digital caliper); height (*A*, cm, with Pilot® ruler), tap root length (*LR*, cm, with Pilot® ruler); aerial (*PSA*, g, scale model Scout, Ohaus® brand), root (*PSR*, g) and total (*PST*, g) dry weights. The morphological indices evaluated were:

shoot:root ratio (*PSA*/*PSR*), slenderness coefficient (*CE*), Dickson index (*ICD*) and lignification index (Prieto *et al.*, 2009). As a reference for values of quality indicators in national conifers, those of Table 1 were used.

Table 1. Values to qualify the quality of plants with normal growth in forest species.

Variable	**Quality**		
	Low	**Medium**	**High**
Dc (mm)	<2.5*	2.5-3.9	≥4.0
A (cm)	<10.0	10.0-14.9	15.0-25.0
CE	>8.0	8.0-6.0	<6.0
RA/*LR*	>2.5	2.1-2.5	≤2
PSA/*PSR*	>2.5	2.1-2.5	1.5-2.0
ICD	<0.2	0.2-0.4	≥0.5

Sources: Conafor (2009), Prieto *et al*. (2009) and Sáenz *et al*. (2014).

Dc =Caliper; *A* = Height; *CE* = Slenderness coefficient; *RA*/*RL* = Root height/length ratio; *PSA*/*PSR* = Shoot:root ratio; *ICD* = Dickson index.

Potential root growth

For the potential root growth test, when the plants reached nine months in the nursery, nine of each of the six treatments were randomly taken, and they were transplanted into pots (5 L) with a mixture of equal parts of agrolite, vermiculite and peat moss. The pots were placed in a greenhouse, according to a randomized complete block design. They were irrigated to maintain the substrate at field capacity. After four weeks, the root balls were extracted and the new roots (white, turgid, ≥1 cm long) were counted.

Field Survival Test

In September, after nine months in the nursery, the trees were planted in the aforementioned reforestation areas; Table 2 indicates the characteristics of the two chosen sites. In each one, 40 plants were established per species-treatment combination, with 4 repetitions (10 plants) and planted in a frame at a distance of 1.5 m. In total, 480 plants were planted. Survival was evaluated at 3, 6, 9, and 12 months.

Table 2. Characteristics of the sites where the *Pinus patula* Schltdl. & Cham. and *P. oaxacana* Mirov plantation was established in the community of *Ixtlán de Juárez*, *Oaxaca*, Mexico.

Characterístics	Site 1	Site 2
Aspect	South	North
Slope (%)	45	32
Altitude (masl)	2 165	2 562
Coordinates (degrees)	17.362448° -96.493407°	17.353672° -96.475008°

Statistical analysis

For the evaluation of plant quality in the nursery, a completely randomized experimental design was used, with a 2×3 factorial arrangement, with two species levels and three fertilization levels. The statistical model was:

$$Y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} \quad (1)$$

Where:

Y_{ijk} = Response variable

μ = General mean

τ_i = Effect of the *i*-th species factor level

β_j = Effect of the *j*- th fertilization factor level

$(\tau\beta)_{ij}$ = Interaction between the *i*-th species factor level and the *j*-th fertilization factor level

ε_{ijk} = Experimental error

The effect of the factors and their interactions on the morphological variables evaluated were tested using an analysis of variance (ANOVA) with the GLM procedure of the statistical analysis package SAS (2002). Effects were considered significant when $p<0.05$. Tukey's post-hoc test ($\alpha=0.05$) was used to test the differences between the treatment means of the factors that were significant.

The differences in survival in the field between the treatments and between the species were examined with the Log-Rank test, by the Kaplan-Meier method (Kaplan and Meier, 1958). For this, the status of each plant (alive or dead) at the end of the evaluation period (12 months), as well as its life time (months) was determined. The analysis was done with the LIFETEST procedure from SAS (2002). The survival function is defined as:

$$S(t) = Pr(T \geq t) \qquad (2)$$

Where:

$S(t)$ = Survival function

$Pr(T \geq t)$ = Probability that a death occurs in a time *T* at least as great as time *t* (Kaplan and Meier, 1958).

To estimate the effect of the studied factors, based on the morphological variables as covariates, a Cox proportional hazards regression was applied. The proportional hazards model used was (Cox, 1972):

$$h_i(t) = h_0(t)\, e^{(\beta_1 X_{i1} + \cdots + \beta_k X_{ik})} \qquad (3)$$

Where:

$h_i(t)$ = Risk of death of an individual *i* at time *t*

h_0 = Unspecified risk function

$X_1, \ldots, X_k$ = Covariables

This model estimates a coefficient β for each factor or covariate and tests the null hypothesis that β=0 with the χ^2 statistic. Such coefficient explains the effect of a factor or a covariate in the risk function. The analysis was carried out using the PHREG procedure of SAS (2002).

Results and Discussion

Plant quality evaluation

According to the optimal intervals to qualify plant quality, the variables and indices that are in the medium and high quality interval are: *Dc*, *A* and *ICD*, in both species. The *CE* and *PSA*/*PSR* variables were of low quality (Table 3).

Table 3. Comparison of means of quality indicators, between species and treatments.

Species	***Pinus patula* Schltdl. & Cham.**			***Pinus oaxacana* Mirov**		
	Fertilization level			**Fertilization level**		
Variable	**Low**	**High**	**Trad.**	**Low**	**High**	**Trad.**
Dc (mm)	3.50 b	4.20 a	4.55 a	3.52 b	3.90 ab	4.24 a

A (cm)	29.0 c	33.2 b	41.9 a	28.2 c	30.3 c	30.1 c
LR (cm)	9.64 c	9.87 ab	9.98 a	9.65 c	9.72 bc	9.87 ab
PSA (g)	2.266 c	3.407 ab	3.782 a	1.851 c	2.629 bc	3.415 ab
PSR (g)	0.511 c	0.737 ab	0.837 a	0.403 c	0.563 bc	0.689 ab
PST (g)	2.778 bc	4.105 a	4.620 a	2.255 c	3.193 b	4.144 a
PSA/*PSR*	4.4 a	4.1 a	5.4 a	4.1 a	4.4 a	4.1 a
CE	8.3 ab	8.0 ab	9.2 a	7.8 ab	7.8 ab	7.3 b
ICD	0.22 bc	0.30 a	0.27 abc	0.21 c	0.23 abc	0.30 ab
IL (%)	20.8 c	33.1 a	34.4 a	27.6 b	30.4 ab	31.9 ab

Trad. = Traditional; *Dc* = Caliper; *A* = Height; *LR* = Root length; *PSA* = Shoot dry weight; *PSR* = Root dry weight; *PST* = Total dry weight; *PSA*/*PSR* = Shoot:root ratio; *CE* = Slenderness coefficient; *ICD* = Dickson index; *IL* = Lignification index. Values with different letters in the same row present significant differences among themselves ($p<0.05$) with the Tukey test.

The *Dc* had the best values, in both species, when the high and traditional nursery dose fertilizations were applied (Table 3). A plant with $Dc \geq 3.5$ mm and $A>28$ cm was produced, values within the range of the Mexican Standard for the Certification of the Operation of Forest Nurseries (Secretaría de Comercio y Fomento Industrial, 2016). Levy and McKay (2003) consider *Dc* as the most reliable indicator of performance in the field. The *Dc* influences robustness, which is associated with vigor and survival (Tsakaldimi *et al.*, 2013). A plant with higher *Dc* is better lignified, has carbohydrate reserves, more buds for regrowth and a more developed root (Rodríguez-Trejo, 2008).

For *A*, in both species and fertilization treatments, quality is high (Table 3). *P. patula* produced with the high and traditional fertilizations had the highest *A*. The plant with the highest *A* (15.0-25.0 cm) is a better competitor in understory sites, but is exposed to greater water stress and lower survival than the taller plant small under adverse conditions (Grossnickle and MacDonald, 2018).

In *P. pseudostrobus* Lindl. *Dc* of 5 mm and *A* of 22 to 25.5 cm have been recorded (Aguilera-Rodríguez *et al.*, 2016) and *Dc* of 3.8 mm and *A* of 27.9 cm (Sáenz *et al.*, 2014). In turn, Aguilera-Rodríguez *et al.* (2021) and González *et al.* (2017) indicated for *P. patula A* of 22 to 30 cm, 26.8 cm and 20 to 30 cm, and *Dc*≥4 mm, 4.12 mm and 3.19 mm respectively, figures similar to those of the present study.

The *LR* had values from 9.6 to 10 cm, the highest were found in *P. patula* with the traditional and high fertilizations, and in *P. oaxacana* with the traditional one, significantly different from the other treatment combinations (Table 3). This variable is restricted by the type and size of the container (González *et al.*, 2017). The *PSA* and *PSR* obtained for *P. patula* with high and traditional fertilization and in *P. oaxacana* with high fertilization were the highest (>3.40 g and >0.68 g), indicating that the plants produced high above-ground biomass and little underground biomass, which is largely due to the size of the container (González *et al.*, 2017).

In *P. oaxacana*, the *CE* values in the three fertilization schemes are between 7.3 and 7.9, so the plant is of medium quality; for this species, Ávila-Angulo *et al.* (2017) reported *CE* from 4.5 to 4.7, low values that are attributed to the *Dc* two times higher than those of the present work. In *P. patula*, the *EC* in the three fertilization schemes are between 8.0 and 9.2, so these plants are of low quality. Aguilera-Rodriguez *et al.* (2021), found in this species an CE<6, high quality, but in this study it resulted in low quality, which is due to the large *A* found in the species, since the higher *A* the *CE* decreases.

The *PSA*/*PSR* ratio for *P. oaxacana* and *P. patula* was 4.1 to 5.4, without significant differences between species or treatments. For both and the fertilization schemes used, the plant was of low quality (*PSA*/*PSR*>2.5, Table 3). With low *PSA*/*PSR*, the plant has a better chance of surviving, since its absorption of water and nutrients improves, but it transpires less (Escobar-Alonso and Rodríguez-Trejo, 2019).

The species, fertilization and their interaction had significant effects (p<0.05) on *A*, *CE* and lignification index. The combination of *P. patula* factors and the nursery's traditional fertilization scheme showed the best values in most of the morphological variables evaluated.

In *P. oaxacana*, the best *ICD* (0.30) was found with traditional fertilization, in *P. patula* this happened with high fertilization (0.30). The results, all the species and fertilization schemes yield a medium quality plant (Table 1). In *P. oaxacana*, Ávila-Angulo *et al.* (2017), refer *ICD* from 1.1 to 1.3, but in *P. patula*, *ICD* of 0.47-0.55, 0.26-0.58 and 0.23-0.25 have been obtained (González *et al.*, 2017; Aguilera-Rodríguez *et al.*, 2021). Values close to 1 indicate balance and balance between shoot and root (Ávila-Angulo *et al.*, 2017).

In both species, the application of high and traditional fertilizations produced *IL*>30 %. Buendía *et al.* (2016) evaluated *P. leiophylla* Schiede ex Schltdl. & Cham. and found an *IL* of 30.9 %. This value is similar to those of the present study. This index estimates the degree of robustness that is needed for the plant to withstand water stress at the plantation site (Prieto *et al.*, 2009).

Potential root growth

Both species had significant differences (p≤0.05) in the number of new roots between fertilization treatments. In *P. oaxacana*, 32, 51 and 74 new roots were counted in the low, high and traditional fertilizations, while in *P. patula* there were 27, 54 and 75. The treatments with higher fertilization have more micronutrients such as Zn, than contributes to the synthesis of indoleacetic acid, which promotes rooting (Alcántar *et al.*, 2016). A greater number of roots in this test denotes more vigor in the tree to acclimatize to the plantation site, especially if it is limiting in humidity (Landis *et al.*, 2010), such as the southern exposure, with respect to the north.

Field survival assessment

The Long-Rank test applied to evaluate survival by species (Figure 1a), did not show significant differences (p=0.7604), since *P. oaxacana* showed a mean survival of 47.9 % and *P. patula* of 47.0 %. The absence of differences is attributed to the fact that both species are native and develop naturally in the forests of this community, and to the fact that they were produced in a similar way in the nursery.

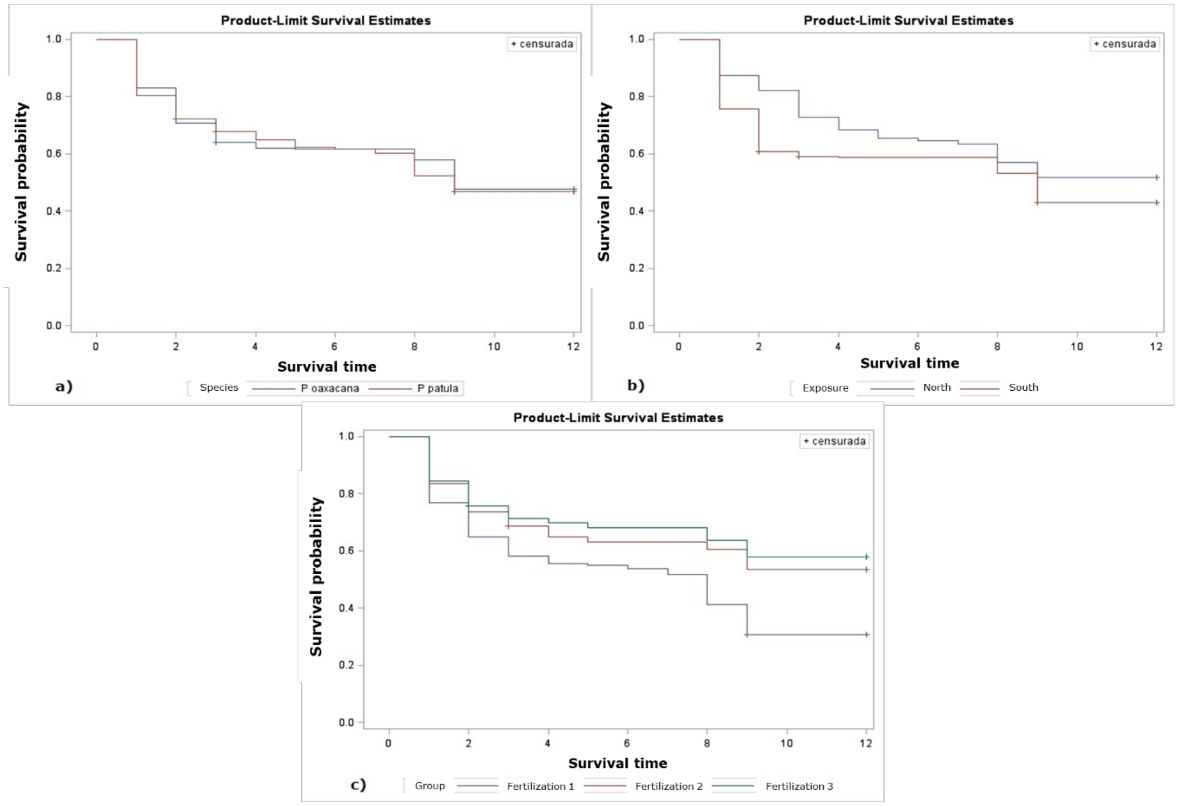

Figure 1. Estimated survival function [S(*t*)] for: a) the two species evaluated in reforestation at both plantation sites, b) the two species, evaluated by aspect in which they were planted, and c) the three fertilization. Schemes at the nursery

For the aspect variable, with the same statistical test, there were significant differences (p=0.0266); the southern aspect (site 1) showed lower survival (43.3 %) (Figure 1b). On the contrary, the northern aspect (site 2) revealed higher survival (51.6 %). Robles *et al.* (2017) reported the effect of aspect (p=0.0222) on the survival of *P. montezumae* Lamb. with higher values in northern aspect (88.7 %), compared to the south (83.3 %). In the northern hemisphere, the southern aspect receives more solar radiation during the year, for which a higher temperature and less available soil moisture prevail. In contrast, the northern aspect is wetter, with lower temperatures and higher biomass (Griffiths *et al.*, 2009).

The Long-Rank test to assess survival by fertilization scheme (Figure 1c) showed significant differences (p<0.0001). The contrasts between low fertilization schemes *vs.* high and low *vs.* traditional, they also had them (p<0.0001 and p<0.0001) (Figure 1c). In the corresponding to high fertilization *vs.* traditional, there were not verified (p=0.4398). The high fertilization showed a survival of 53.75 %, and the traditional, 58.13 %. Low fertilization had the lowest survival (30.63 %).

The favorable survival response for the high and traditional fertilization schemes can be explained by the morphophysiological characteristics that the plants developed in the nursery, as a result of such fertilizations. The higher doses of N in these treatments -an element that makes up proteins and particularly influences growth to reach dimensions, such as *Dc*, related to quality- as well as that of K -which participates in osmotic regulation and in the opening and closing stomatal, and thus contributes to better resist low temperatures and humidity limitations (Rodríguez, 2008; Alcántar *et al.*, 2016)- contributed to the higher survival values.

Hazard analysis

The Cox proportional hazards model was significant for the analyzed data set and studied variables (p<0.0001). Aspect, fertilization, *Dc* and *A* had a significant effect on the risk function (p≤0.05), while the *PST* and *ICD* variables did not show a significant effect (p>0.05) on it (Table 4).

Table 4. Results of the proportional hazards regression.

Analysis of the maximum likelihood estimator							
Parameter/Variable		**GL**	**Estimator**	**Standard error**	**χ^2**	**Pr > χ^2**	**Risk ratio**
Species	*Pinus patula* Schltdl. & Cham.	1	0.07828	0.25206	0.0965	0.7561	1.081
Aspect	South	1	0.52910	0.12655	4.3697	0.0366	1.697
Fertilization scheme	F 1	1	0.52071	0.15025	12.0102	0.0005	1.683
	F 2	1	0.19306	0.15025	1.6510	0.1988	1.213
Root collar diameter (*Dc*)		1	-1.02674	0.29555	12.0686	0.0005	0.358
Height (*A*)		1	0.0362	0.02604	1.9332	0.041	1.037
Total dry weight (*PST*)		1	0.11432	0.30894	0.1369	0.7113	1.121
Dickson index (*ICD*)		1	-0.59728	2.86908	0.0433	0.8351	0.55

The plants with low fertilization showed a positive estimator and a risk ratio of 1.683, which indicates that taking a plant to the field with such a scheme increases the risk of death by 68.3 % during the first months after planting, with respect to high dose and traditional fertilization. Fertilizing the plant in the nursery favors its morpho-physiological condition, which contributes to improving its quality, that is, its survival and initial growth (Alcántar *et al.*, 2016).

The Cox risk analysis showed that the *Dc* had a significant effect on the risk function, with a negative β estimator and a risk ratio of 0.358. This means that the 1 mm increase in the *Dc* of the plants reduces the risk of death up to 64.1 %, [$100(1-e^{-1.02674})$], provided that the other variables remain constant. According to Levy and McKay (2003), plants with larger *Dc* survive and grow better than those with smaller *Dc*. The *Dc* of *P. pseudostrobus* it is related to its survival in the field; an increase of 1 mm in *Dc* reduces the risk of death up to 66.8 % (Sigala *et al.*, 2015), a value similar to that of the present study. Authors such as Tsakaldimi *et al.* (2013) concluded that for *Pinus* and other species, the *Dc* influences survival during the first months of establishment. A similar trend has been observed in *P. cooperi* C. E. Blanco and *P. engelmannii* Carrière in the state of *Durango*, for *A* (Prieto *et al.,* 2018).

Conclusions

The results for the morphological quality indicators, such as *A* and *Dc*, validate the traditional fertilization of the nursery for both species. It is also suggested that high fertilization is equally recommended for *P. patula*. In the present work, the classic canon of the importance of producing a robust plant, with good *Dc*, is highlighted.

Field survival at 12 months was acceptable, greater than 50 % for both species, due to the effect of high and traditional fertilization. However, the former may be cheaper. Quality standards must be established by species, but also by provenance and plantation site, since under different environmental conditions, such as those given by different aspect, plants with the same quality attributes will have different survivals.

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Conflict of interests

The authors declare no conflict of interest.

Contribution by author

Martin Paz Paz: design and installation of experiments, cultivation of the plant, application of treatments, measurements, statistical analysis and writing of the manuscript; Dante Arturo Rodríguez Trejo: design and supervision on: installation of the experiment, cultivation of the plant, application of treatments and revision of the manuscript; Antonio Villanueva Morales: supervision of the experimental design, general monitoring, statistical analysis and revision of the manuscript; Ma. Amparo Máxima Borja de la Rosa: general monitoring and review of the manuscript.

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