

Article

Nursery Production of *Pinus engelmannii* Carr. with Substrates Based on Fresh Sawdust

María Mónica González-Orozco ¹, José Ángel Prieto-Ruiz ^{2,*}, Arnulfo Aldrete ³,
José Ciro Hernández-Díaz ⁴, Jorge Armando Chávez-Simental ⁴ and Rodrigo Rodríguez-Laguna ⁵

¹ Programa Institucional de Doctorado en Ciencias Agropecuarias y Forestales, Universidad Juárez del Estado de Durango, Constitución 404 sur Zona centro, Durango 34000, Mexico; m_gonzalez@ujed.mx

² Facultad de Ciencias Forestales, Universidad Juárez del Estado de Durango, Río Papaloapan y Boulevard, Durango S/N Col. Valle del Sur, Durango 34120, Mexico

³ Colegio de Postgraduados, Campus Montecillo, Carretera México-Texcoco Km 36.5, Montecillo, Texcoco, Estado de México 56230, Mexico; aaldrete@colpos.mx

⁴ Instituto de Silvicultura e Industria de la Madera, Universidad Juárez del Estado de Durango, Boulevard del Guadiana 501, Ciudad Universitaria, Torre de Investigación, Durango 34120, Mexico; jciroh@ujed.mx (J.C.H.-D.); jorge.chavez@ujed.mx (J.A.C.-S.)

⁵ Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo, Universidad Km. 1, Ex Hacienda de Aquetzalpa, Tulancingo, Hidalgo 43600, Mexico; rodris71@yahoo.com

* Correspondence: jprieto@ujed.mx; Tel.: +52-618-827-12-20

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Abstract: Substrate is a factor that significantly influences the quality and production costs of nursery seedlings. The objective of this study was to evaluate combinations of peat moss, composted pine bark, and fresh pine sawdust in order to identify the proportions that favour the quality of *Pinus engelmannii* Carr. seedlings and minimise the production costs in the nursery. Substrates were formed using mixtures of peat moss (15% to 50%), composted pine bark (15% to 50%) and fresh pine sawdust (20% to 70%), with 2, 4 and 6 g L⁻¹ of controlled release fertilizer (Multicote[®], Haifa, Israel). A completely randomised experimental design with a factorial arrangement of 7 × 3 was used. The evaluated factors are root collar diameter, biomass, N-P-K content, and production costs of the substrates which were determined based on the container volume and three commercial quotations. Significant differences were found in root collar diameter and biomass, highlighting the treatments using 50% to 70% sawdust with 6 g L⁻¹ of fertilizer. Assimilated values of N-P-K were acceptable in all treatments with 4 and 6 g L⁻¹ of fertilizer. In the substrates with high percentages of sawdust, seedlings with morphological characteristics and nutritional levels within the values recommended for conifers were produced. In addition, it was possible to reduce the production cost of the substrates by up to 67%.

Keywords: composted pine bark; fresh pine sawdust; seedling quality; peat moss; *Pinus engelmannii* Carr.

1. Introduction

Mexico has 42% of the world's total number of *Pinus* species, of which 47% are found naturally in the state of Durango in northwest Mexico [1]. Within this great diversity, *Pinus engelmannii* Carr. stands out due to its wide distribution in semi-dry and temperate forests of the Sierra Madre Occidental, which is a system of mountains in the west of Mexico. This species is found on hillsides of between 1900 and 2700 m in altitude, in areas with deep or poor and rocky soils [2]. During its initial growth, *P. engelmannii* has increases in diameter in relation to height [3]. From an economic point of view,

this species is important due to the characteristics of the wood, which makes it widely used in reforestation programmes and commercial forest plantations. Hence, it is the second most produced species in forest nurseries in northern Mexico [4–6].

In the nursery, *P. engelmannii* reproduces by seed, and is generally planted in polystyrene containers or polyethylene tubes, with volumes of 170 to 220 mL, with growth media based on peat moss, pine bark, perlite and vermiculite [5,7]. The seedling quality varies in the different nurseries, hence, viable culture alternatives are sought that allow the production of individuals with suitable characteristics; the characteristics include: a well-developed root system, root collar diameter greater than 5 mm, balance between stem-root, foliage adapted to weather conditions, adequate carbohydrate reserves, and sufficient mineral nutrition [8,9]. These attributes, together with mineral nutrition, influence the growth and adaptation of individuals at the plantation site [10]. Therefore, it is important that fertilization is adequate, as it will promote growth and strengthen tolerance to stress in unfavourable environments [11].

Another important component in the seedling production process in the nursery is the substrate, since the properties of the constituent materials have an effect on seedling growth and future development [12]; and the materials are related to substrate quality [13]. In addition, substrate has a direct relationship with the costs of plant production. Research on the substitution of peat by local substrates, as a culture medium in nurseries, is of great interest [14–17], since this material has a significant influence on the final cost of seedling production [9]. Importantly, excessive extraction of peat in ecosystems with wetlands, where it is obtained, causes great environmental damage [14,18].

Recent studies have shown that fresh sawdust, as a component of the culture medium, serves as an alternative in the production of forest species, such as *Cedrela odorata* L. [19], *Pinus greggii* Engelm. [20], *Pinus montezumae* Lamb. [21] and *Pinus pseudostrobus* Lindl., among others [16,22]. However, while these types of studies have evaluated a few *Pinus* species that are located in the centre of the country, to our knowledge, there have been no such studies of species such as *P. engelmannii* in northern Mexico.

These considerations support the objective of evaluating combinations of peat moss, composted pine bark, and fresh pine sawdust, in combination with three doses of controlled-release fertilizer, in order to identify the proportions that favour seedling quality and minimise costs of production in the nursery. We propose the hypothesis that substrates which include high proportions of fresh sawdust in combination with controlled release fertilizers, allow the production of *P. engelmannii* seedlings with adequate morphological characteristics for optimal development, while reducing production costs.

2. Materials and Methods

2.1. Study Site

This study was carried out in the “General Francisco Villa” Forest Nursery, which is located at coordinates 23°58′20.38″ N and 104°35′55.83″ W, at an altitude of 1875 m, in the City of Durango, State of Durango, Mexico. The experiment was established in a greenhouse covered with 720 gauge plastic and 50% shade mesh, with overhead, lateral and frontal ventilation, and with an automated irrigation system having micro-sprinklers.

2.2. Establishment of the Experiment

The production seedling cycle was from 15 September 2015 to 20 June 2016 (10 months), and in this period the temperature and relative humidity were monitored (Figure 1). The sowing was performed in polystyrene containers of 77 cavities with 170 mL per cavity, using seeds of *P. engelmannii* that had been collected in the Coyote de Calaveras, an area which is located in Durango. Before sowing, the seeds received a pre-germinative treatment (soaking in water for 24 h at room temperature) and then they were disinfected for 5 min in a solution of 90% water and 10% chlorine. Finally, 2.5 g L^{−1} of fungicide (Captán® *N*-trichloromethylthio-4-cyclohexene-1,2-dicarboximide, Industrial engineer, Mexico City, Mexico) dissolved in water was applied.

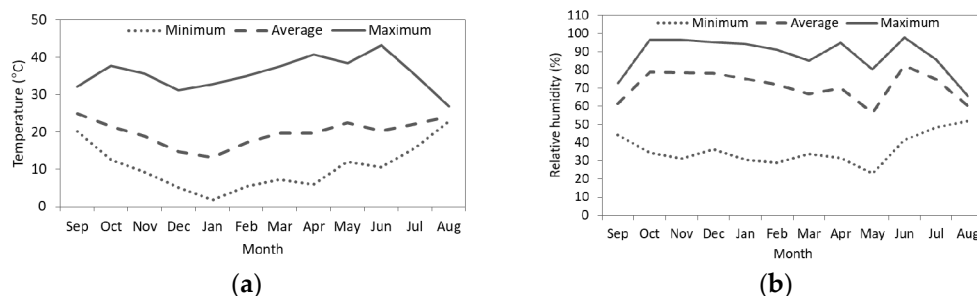


Figure 1. Temperature (a) and relative humidity (b) recorded during the production of *Pinus engelmannii* from September 2015 to June 2016.

2.3. Substrate and Fertilizer

Seven substrates composed of peat moss, composted bark of *Pinus douglasiana* Martínez, and fresh pine sawdust were evaluated, three weeks after being obtained from the sawing of logs of *Pinus engelmannii* Carr., *Pinus cooperi* Blanco, and *Pinus durangensis* Martínez (Table 1). Multicote® 18 N - 6 P₂O₅ + - 12 K₂O + 2 Mg + ME fertilizer (Haifa Chemicals Ltd., Hifa, Israel) was used, with 8 to 9 months of nutrient release, in three doses: 2 g L⁻¹ (M2), 4 g L⁻¹ (M4) and 6 g L⁻¹ (M6). The said doses of fertilizer were incorporated into the substrate when preparing the mixture. With the interaction of the seven substrates and the three doses of fertilization, 21 treatments were formed, which were identified using the following keys: S1-M2, S1-M4, S1-M6, S2-M2, S2-M4, S2-M6, S3-M2, S3-M4, S3-M6, S4-M2, S4-M4, S4-M6, S5-M2, S5-M4, S5-M6, S6-M2, S6-M4, S6-M6, S7-M2, S7-M4 and S7-M6, where S refers to the substrate and M refers to the Multicote® fertilizer in its different mixtures and doses, respectively.

Table 1. Substrates evaluated in the production of *Pinus engelmannii* seedlings in the nursery, during the crop cycle of September 2015 to June 2016.

Code	Substrate
S1 (Control)	50% Peat moss + 50% Composted pine bark
S2	40% Peat moss + 40% Composted pine bark + 20% Fresh pine sawdust
S3	35% Peat moss + 35% Composted pine bark + 30% Fresh pine sawdust
S4	30% Peat moss + 30% Composted pine bark + 40% Fresh pine sawdust
S5	25% Peat moss + 25% Composted pine bark + 50% Fresh pine sawdust
S6	20% Peat moss + 20% Composted pine bark + 60% Fresh pine sawdust
S7	15% Peat moss + 15% Composted pine bark + 70% Fresh pine sawdust

In addition to the doses of controlled-release fertilizer, the seedlings in all of the treatments were irrigated using water-soluble fertilizers (Poly-Feed® Foliar, Haifa, Israel) during the production cycle, according to the growth phases of the seedlings. In the first or establishment phase (8%-52%-17%, N-P-K), the dose was from 40 to 70 ppm of nitrogen with a duration of 2.5 months. In the rapid growth phase (20%-9%-20%, N-P-K), the dose was 100 to 200 ppm of nitrogen with a duration of 2.5 months and, in the last or pre-conditioning phase (4%-25%-40%, N-P-K), the dose was 20 to 40 ppm of nitrogen, which lasted for two months. The three doses were applied during irrigation by using an automated system. This regimen of foliar fertilization is the one used in the Francisco Villa Nursery, combined with 3 g L⁻¹ of Multicote®, when it is used as a substrate of 50% moss peat + 50% composted pine bark. In addition, the presence of pests and diseases was prevented with the application of insecticides (LORSBAN®, Tlaxcala, Mexico, CONFIDOR®, Leverkusen, Germany, ENGEO®, Mexico City, Mexico) and fungicides (CAPTAN®, Mexico City, Mexico, TECTO®, Mexico City, Mexico, DEROSAL®, Leverkusen, Germany) every 4 days.

2.4. Experimental Design

A completely randomised experimental design was used with a factorial arrangement of 7×3 , with seven substrates and three doses of fertilization. The experimental unit consisted of 12 seedlings with four replications per treatment, with a total of 1008 seedlings being evaluated in the trial.

2.5. Variables Evaluated

2.5.1. Physical and Chemical Characteristics of Substrate Mixtures

The physical characteristics that were determined for the substrates were: aeration porosity (%), humidity retention porosity (%), and total porosity (%), using the method described by Landis [23], and the chemical properties were: pH and electrical conductivity (dS m^{-1}), which were based on NOM-021-RECENAT-2000. These studies were carried out at the beginning of production, in the Environmental Sciences Laboratory, of the Integral Rural Centre for Interdisciplinary Research for Integral Rural Development (CIIDIR is the Spanish-language acronym), which is a Durango-based unit of the National Polytechnic Institute (IPN is the Spanish-language acronym).

2.5.2. Morphological Variables

When the seedlings were ten months of age, the root collar diameter (mm) was measured with a digital vernier caliper, together with the dry aerial biomass, dry root biomass, and dry total biomass (g). To determine the biomass, the plants were dehydrated in a drying oven at 70°C for 72 h, until reaching a constant weight. Then, the samples were weighed on an Ohaus® (Ohaus of Mexico, Mexico City, Mexico) analytical balance with an accuracy of 0.0001 g.

2.5.3. Concentrations of Nitrogen (N), Phosphorus (P) and Potassium (K)

Representative samples of dry foliage were integrated to determine the content of N, P and K; these consisted of needles from the middle part of each seedling, up to 5 g per treatment, with three repetitions. N content was determined by using the *Kjeldahl* method [24]. P content was determined by complex determination of yellow colour production of vanadomolybdate reagent in reaction with phosphates, and K content was determined by using atomic emission (the samples were digested using 60% nitric acid and 40% perchloric acid) [25]. With the obtaining of the concentration of each element, vector nomograms were constructed, based on the weight of 100 needles of each treatment [26].

2.5.4. Cost of the Substrate

The cost of the substrate was determined based on the volume of the cavity (170 mL) of the container, and was valued in US dollars based on three commercial quotations (Date: 15 March 2018).

2.6. Statistical Analysis

The data of the morphological variables were evaluated by the non-parametric statistical test of Kruskal-Wallis, due to the non-compliance of the normality assumptions; therefore, the Bonferroni-Dunn means separation test ($p \leq 0.05$) was used. The data were analyzed using the R statistical software version 3.2.3, Vienna, Austria [27].

3. Results

3.1. Physical and Chemical Characteristics of Substrate Mixtures

Aeration porosity ranged from 23.6% in S6 to 29.2% in S1; humidity retention porosity varied from 33.6% in S1 to 49.4% in S7; while total porosity presented a range of 62.8% in S1 to 76.3% in S7; in the latter two variables, the porosity increased as the proportion of sawdust increased. Regarding the chemical

characteristics of the substrate mixtures, the pH and electrical conductivity presented slightly higher values in the substrates which included sawdust, in comparison with the control substrate (S1) (Table 2).

Table 2. Physical and chemical properties of the substrates evaluated in the production of *Pinus engelmannii*.

Substrate	Physical (%)			Chemical	
	Aeration Porosity	Humidity Retention Porosity	Total Porosity	pH	Electrical Conductivity (dS m ⁻¹)
S1	29.2	33.6	62.8	4.07	0.06
S2	28.1	39.2	67.3	4.47	0.08
S3	25.5	41.3	66.8	4.54	0.09
S4	26.4	40.6	67.0	4.65	0.09
S5	25.6	39.8	65.4	4.68	0.09
S6	23.6	46.1	69.7	4.82	0.07
S7	26.9	49.4	76.3	4.74	0.08
RV	25 to 35	25 to 55	60 to 80	5 to 6.5	<1.0

S1: 50% peat moss + 50% composted pine bark, S2: 40% peat moss + 40% composted pine bark + 20% fresh pine sawdust, S3: 35% peat moss + 35% composted pine bark + 30% fresh pine sawdust, S4: 30% peat moss + 30% composted pine bark + 40% fresh pine sawdust, S5: 25% peat moss + 25% composted pine bark + 50% fresh pine sawdust, S6: 20% peat moss + 20% composted pine bark + 60% fresh pine sawdust, S7: 15% peat moss + 15% composted pine bark + 70% fresh pine sawdust. RV: Recommended values [23,28].

3.2. Morphological Variables

3.2.1. Root Collar Diameter

The root collar diameter was not influenced significantly by the substrate or fertilizer doses. The interaction of the factors presented significant differences, with the highest values being related to the seedlings that were produced in the treatments S5-M2 (7.41 mm) and S4-M4 (7.35 mm) (Table 3, Figure 2).

3.2.2. Dry Aerial Biomass

The dry aerial biomass did not present significant differences at the substrate level, while at the fertilizer level it was significantly higher at the 6 g L⁻¹ dose with 4.74 g. The interaction of the factors showed significant differences, highlighting the S7-M6 treatment (5.40 g) (Table 3, Figure 2).

3.2.3. Dry Root Biomass

The dry root biomass showed significant differences at the substrate level, in which the substrate with the highest proportion of sawdust S7 (1.35 g) was notable, while the effect of the fertilising factor was non-significant. The interaction of the factors presented highly significant differences, and the treatment with the highest value was S7-M6 (1.56 g) (Table 3, Figure 2).

3.2.4. Dry Total Biomass

The dry total biomass did not show significant differences in its influence over the substrate, while the fertilizer significantly influenced with the dose of 6 g L⁻¹ with 5.96 g in the upper level. The substrate and fertilizer interaction was significant, within which the treatment S7-M6 (6.96 g) stood out. In all the morphological variables that were evaluated, the treatments that included sawdust were superior to the control, whereas the control only included peat moss and composted pine bark (Table 3, Figure 2).

Table 3. Significance values (p value) based on the response of the substrate, the fertilizer and its interaction, in the variables evaluated in *Pinus engelmannii*, with the non-parametric Kruskal-Wallis test.

Variable	Substrate	Fertilizer	Substrate $\times$ Fertilizer
Root collar diameter	0.3276 ^{ns}	0.3506 ^{ns}	<0.0001 ^{***}
Dry aerial biomass	0.6821 ^{ns}	<.0010 ^{**}	0.0010 ^{**}
Dry root biomass	0.0223 [*]	0.4116 ^{ns}	<0.0010 ^{**}
Dry total biomass	0.7577 ^{ns}	0.0057 ^{**}	0.0060 ^{**}

Significant differences are denoted by: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, and ^{ns}: non-significant.

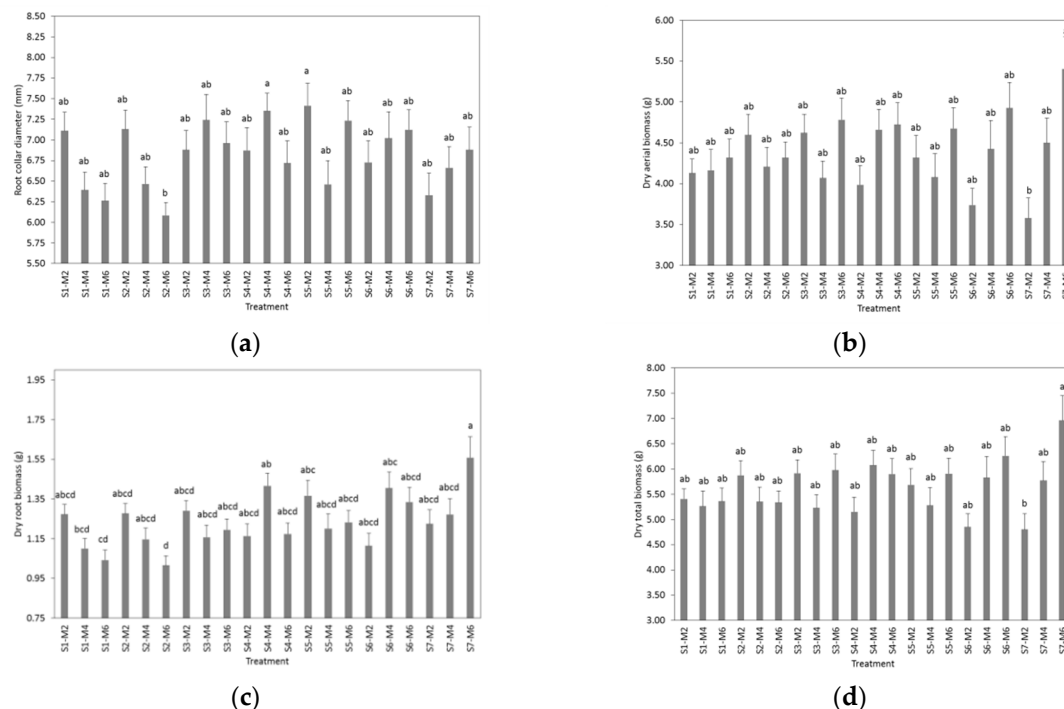


Figure 2. Variables evaluated in *Pinus engelmannii*: Root collar diameter (a); dry aerial biomass (b); dry root biomass (c) and dry total biomass (d). Differences between letters indicate significant differences between treatments (Bonferroni, $\alpha = 0.05$).

3.3. Nutritional Analysis of the Foliage

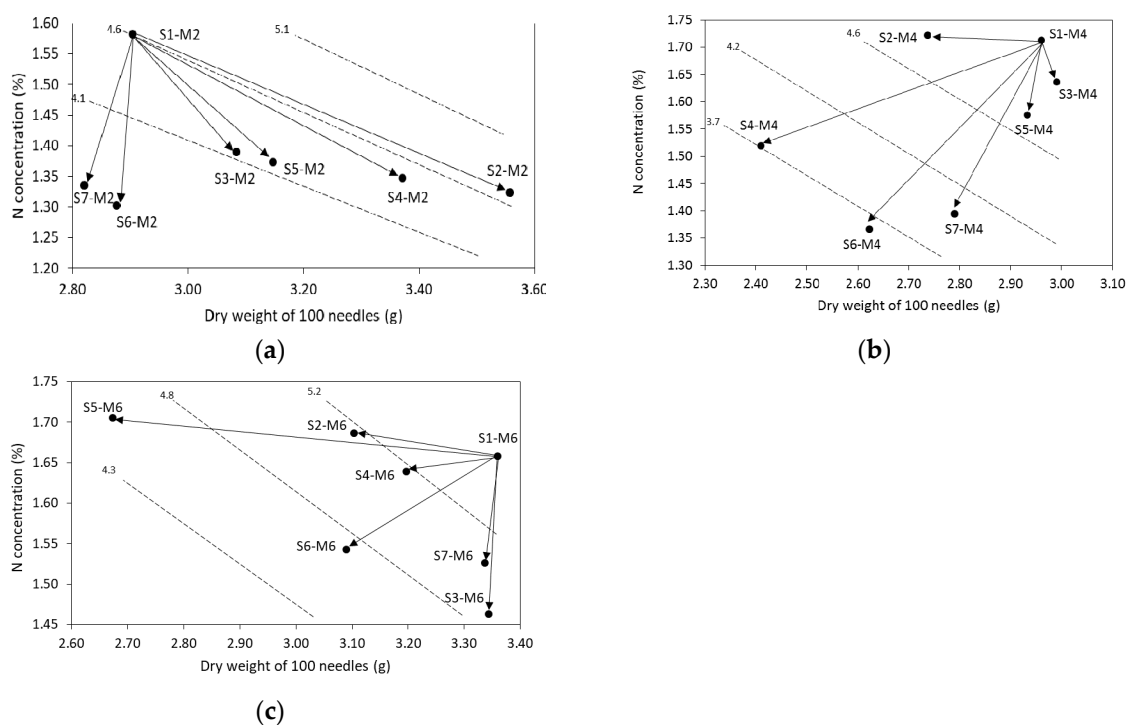
In the concentrations of N, P and K, no significant differences were found between treatments (Table 4). Despite this, relationship of concentrations and needles weight generated variable responses in the vector nomograms of each element, based on the substrates and doses evaluated.

3.3.1. Nitrogen (N)

The nomogram of N with 2 g L^{-1} Multicote[®], indicates that the treatments S2-M2, S3-M2, S4-M2, and S5-M2 generated an increase in the biomass of needles, but had a lower concentration of the element with respect to S1-M2 (substrate without sawdust). Meanwhile, the treatments S6-M2 and S7-M2 presented an amount of biomass of needles that was similar to S1-M2, but with a lower concentration of N (Figure 3a).

Table 4. Means of N, P and K concentrations in foliage of *Pinus engelmannii*.

Treatment	N (%)	P (%)	K (%)
S1-M2	1.6 ± 0.01	0.31 ± 0.01	1.3 ± 0.16
S1-M4	1.7 ± 0.15	0.30 ± 0.02	1.2 ± 0.02
S1-M6	1.7 ± 0.03	0.31 ± 0.01	1.2 ± 0.01
S2-M2	1.3 ± 0.17	0.31 ± 0.03	1.2 ± 0.08
S2-M4	1.7 ± 0.11	0.31 ± 0.00	1.1 ± 0.08
S2-M6	1.7 ± 0.02	0.30 ± 0.01	1.2 ± 0.11
S3-M2	1.4 ± 0.04	0.27 ± 0.00	1.2 ± 0.12
S3-M4	1.6 ± 0.10	0.29 ± 0.03	1.1 ± 0.10
S3-M6	1.5 ± 0.20	0.31 ± 0.01	1.2 ± 0.14
S4-M2	1.3 ± 0.12	0.30 ± 0.03	1.5 ± 0.14
S4-M4	1.5 ± 0.17	0.31 ± 0.00	1.2 ± 0.14
S4-M6	1.6 ± 0.08	0.29 ± 0.01	1.4 ± 0.16
S5-M2	1.4 ± 0.05	0.26 ± 0.02	1.1 ± 0.01
S5-M4	1.6 ± 0.04	0.29 ± 0.01	1.1 ± 0.10
S5-M6	1.7 ± 0.16	0.29 ± 0.03	1.2 ± 0.18
S6-M2	1.3 ± 0.01	0.27 ± 0.02	1.3 ± 0.05
S6-M4	1.4 ± 0.07	0.29 ± 0.01	1.1 ± 0.08
S6-M6	1.5 ± 0.14	0.28 ± 0.02	1.3 ± 0.06
S7-M2	1.3 ± 0.05	0.28 ± 0.01	1.2 ± 0.04
S7-M4	1.4 ± 0.07	0.28 ± 0.01	1.2 ± 0.06
S7-M6	1.5 ± 0.04	0.27 ± 0.00	1.2 ± 0.03
p value	0.7543	0.9037	0.9976

**Figure 3.** Nomograms of nitrogen (N) concentration vectors in the foliage of *Pinus engelmannii*, produced on seven substrates with the addition of three fertilization doses: (a) 2 g L^{−1} Multicote[®]; (b) 4 g L^{−1} Multicote[®] and (c) 6 g L^{−1} Multicote[®].

On the other hand, according to the nomogram where 4 g L^{−1} of Multicote[®] was added, the S3-M4 treatment showed a greater increase in the biomass of needles in relation to S1-M4, but had a slight decrease in N; treatments S4-M4, S5-M4, S6-M4 and S7-M4 showed a smaller increase in the

biomass of needles and lower concentration of N than S1-M4; and finally, the S2-M4 treatment showed a lower increase in biomass of needles, and the same concentration of N (Figure 3b).

In the nomogram with 6 g L⁻¹ of Multicote[®], it can be observed that the treatments S3-M6 and S7-M6 present a biomass of needles similar to S1-M6, but with a lower concentration of N. On the other hand, the treatments S4-M6 and S6-M6 show a lower biomass of needles and a lower concentration of N. Finally, treatments S2-M6 and S5-M6 present a lower biomass of needles and a similar concentration of N, compared to S1-M6 (Figure 3c).

3.3.2. Phosphorus (P)

The nomogram with the dose of 2 g L⁻¹ Multicote[®], shows that the treatments S2-M2 and S4-M2 generated a greater increase in the biomass of needles and similar concentration, with respect to the S1-M2. On the other hand, treatments S3-M2 and S5-M2 show a higher biomass of needles, but with a lower concentration of P, while treatments S6-M2 and S7-M2 present a lower biomass of needles and a lower concentration of P (Figure 4a).

On the other hand, in the nomogram with the addition of 4 g L⁻¹ of Multicote[®], only S3-M4 presents a biomass in needles that is similar to S1-M4, but with a lower concentration of P; treatments S5-M4, S6-M4 and S7-M4 show a lower increase in the biomass of needles and a lower concentration than S1-M4; finally, in the treatments S2-M4 and S4-M4 there was a smaller increase in biomass of needles, and a slight increase in the concentration of P (Figure 4b).

In the nomogram with 6 g L⁻¹ of Multicote[®], treatment S3-M6 has generated the same amount of biomass of needles and concentration of P as S1-M6, while S7-M6 shows the same biomass of needles but lower concentration of P; finally, the treatments S2-M6, S4-M6, S5-M6 and S6-M6 have a lower biomass of needles and a lower concentration of P, all in comparison to S1-M6 (Figure 4c).

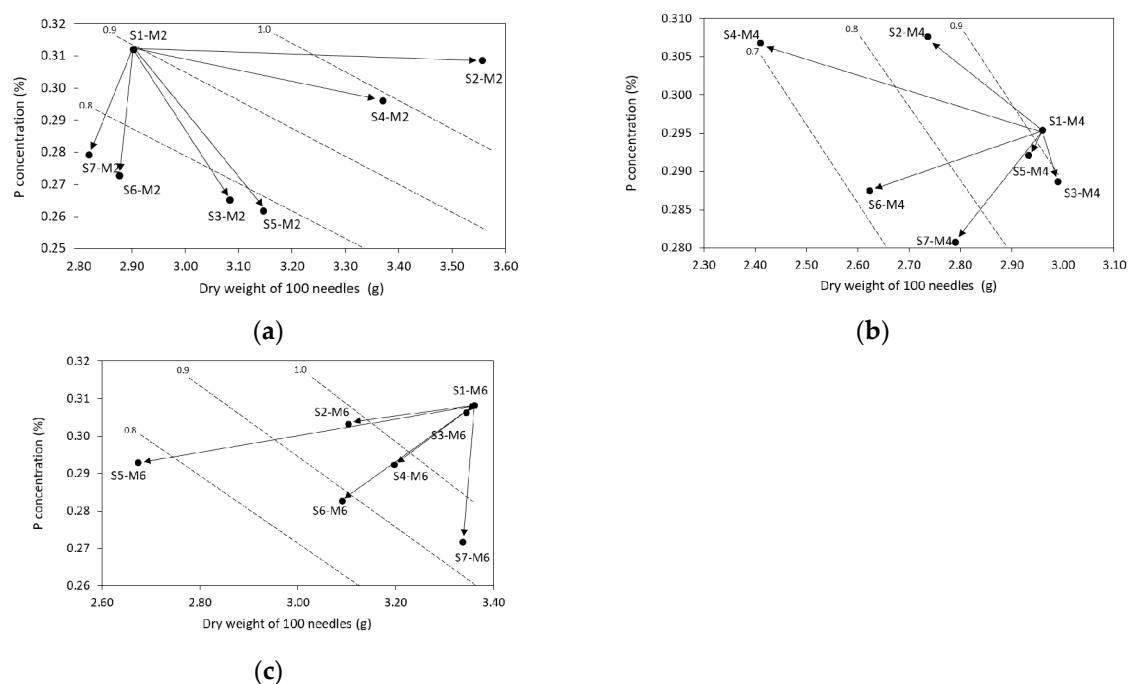


Figure 4. Vector nomograms of the concentration of phosphorus (P) in the foliage of *Pinus engelmannii*, produced in seven substrates with the addition of three doses of fertilization: (a) 2 g L⁻¹ Multicote[®]; (b) 4 g L⁻¹ Multicote[®] and (c) 6 g L⁻¹ Multicote[®].

3.3.3. Potassium (K)

The nomogram with 2 g L⁻¹ of Multicote[®], shows that the treatment S4-M2 generated a greater increase in the biomass of needles and a higher concentration of K with respect to the S1-M2. On the

other hand, the treatments S2-M2, S3-M2 and S5-M2 achieved a greater biomass of needles than S1-M2, but with a lower concentration of K; while the treatments S6-M2 and S7-M2 had a lower biomass of needles and lower concentration of K (Figure 5a).

On the other hand, in the nomogram with the addition of 4 g L^{-1} of Multicote®, only the S3-M4 attained a greater increase of biomass in needles in relation to S1-M4, but with a lower concentration of K; treatments S2-M4, S5-M4 and S6-M4 show a lower increase in the biomass of needles and a lower concentration of K than S1-M4. Finally, treatments S4-M4 and S7-M4 show a lower increase in biomass of needles, but a higher concentration of K (Figure 5b).

In the nomogram with 6 g L^{-1} Multicote®, the treatments S3-M6 and S7-M6 have practically the same amount of biomass and concentration as the S1-M6 treatment, while the treatments S4-M6 and S6-M6 express a lower biomass of needles but with higher concentrations of K; the S5-M6 treatment has a lower biomass of needles and an equal concentration of K. Finally, the S2-M6 treatment presents a lower biomass of needles and a lower concentration of K (Figure 5c).

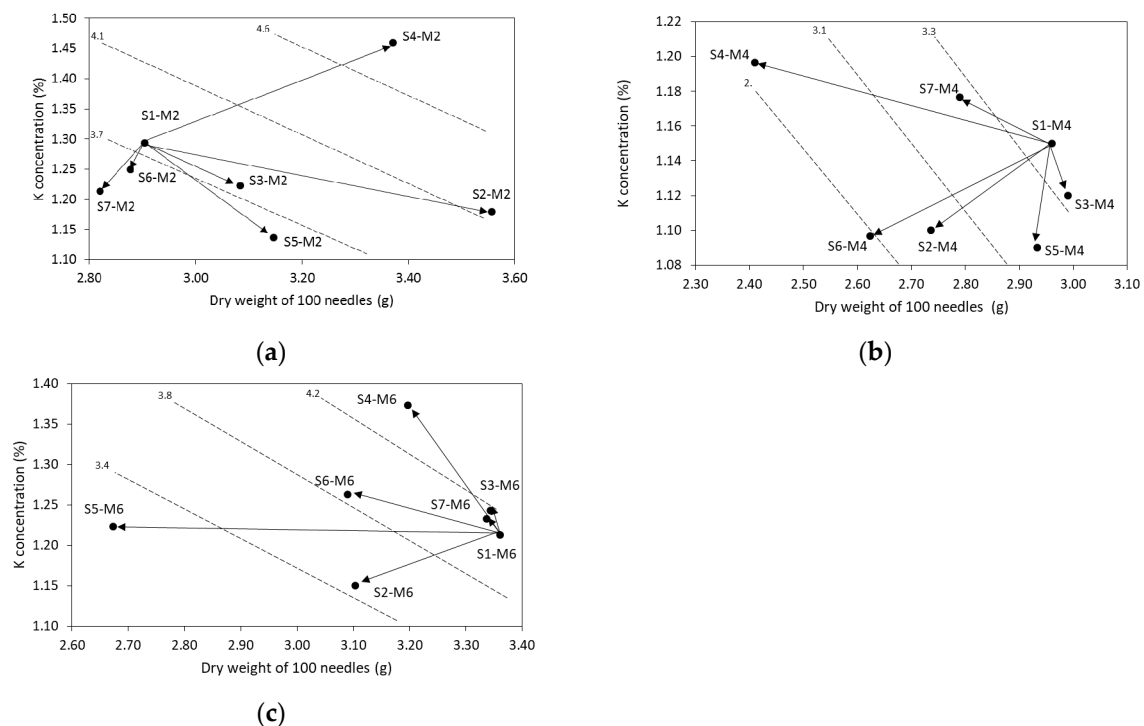


Figure 5. Vector nomograms of the potassium concentration (K) in the foliage of *Pinus engelmannii*, produced in seven substrates with the addition of three doses of fertilization; (a) 2 g L^{-1} Multicote®; (b) 4 g L^{-1} Multicote® and (c) 6 g L^{-1} Multicote®.

3.4. Substrate Cost

The cost per litre of substrate was valued in dollars as follows: S1 = 3.49, S2 = 2.82, S3 = 2.49, S4 = 2.15, S5 = 1.82, S6 = 1.48 and S7 = 1.15 (\$). Based on the previous quotations, the substrates that contain sawdust are between 23% and 67% more economical, in relation to the substrate that contains only peat moss and composted pine bark. With the costs obtained from the substrate, an extrapolation was carried out in a production of 100,000 plants (Figure 6), where the investment in the seedling production by substrates appreciates when including the fresh sawdust.

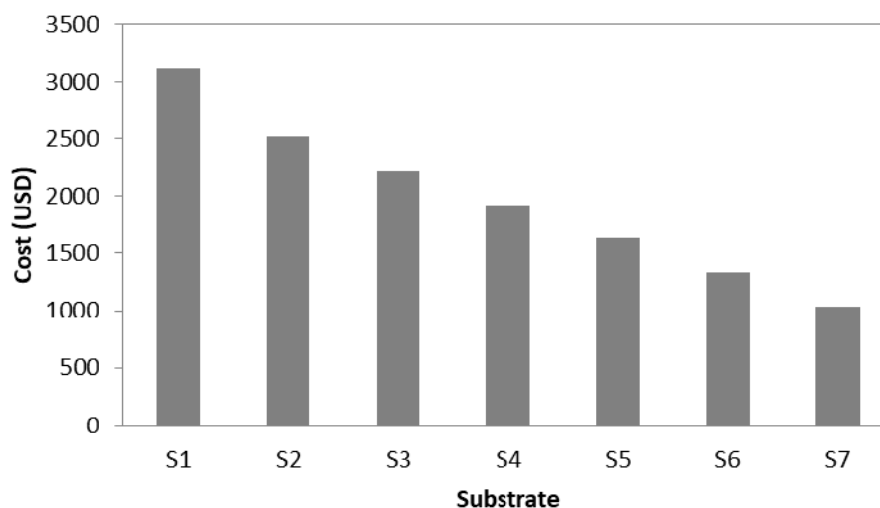


Figure 6. Projected substrate cost in a production of 100,000 seedlings of *Pinus engelmannii*, produced in polystyrene containers with 77 cavities of 170 mL.

4. Discussion

4.1. Physical and Chemical Characteristics of Substrate Mixtures

In general, the substrates that were evaluated in the present study had percentages of moisture retention within the recommended values, which were increasing in the mixtures when increasing the percentage of sawdust, due to the size of the fine particles of that sawdust. As a consequence, aeration porosities were lower in the mixtures with higher percentage of sawdust, without presenting root rot problems due to excess moisture, since the size and composition of the pine bark particles provide a greater flow of air, and greater water filtration [29], although it is inevitable that the porosity decreases with time due to the compaction and settlement of the substrate by way of constant humidity changes [30].

The three porosities that were evaluated are within the recommended ranges for producing coniferous seedlings (Table 1) [23,28]. This indicates that the evaluated substrates retain sufficient moisture, due to the degree of porosity that was determined in this study, which generated a broad root system that favoured the growth of the seedlings, and that root system is expected to help withstand severe growing conditions in the field [15]. Other works in the body of literature agree on the feasibility of including fresh sawdust in substrates when producing *Pinus spp.*, and on which substrates can be formulated regarding their appropriate physical and chemical characteristics [16,17,21].

Because these properties can change according to time and source, they must always be tested for each condition [9], since the physical and chemical properties of organic waste can change according to type and origin [31]. In this case, the materials used in this study did not cause problems of diseases and pests, nor did they cause toxicity in the seedlings when using sawdust without composting.

The pH and electrical conductivity are also important properties, as they influence the availability of nutrients in growth media [32]. On the one hand, the pH presented values with a strong acidity in all of the treatments, but the S6 substrate (pH of 4.82) with sawdust presented a value that was closer to the recommended value (pH of 5). However, pH values increase during the production process, due to watering. Substrates including composted pine bark and sawdust have been found with pH values of 4.3 to 4.7 [21], that is, pH values that are very close to those reported in the present study. In general, pH values within the range of 4.0 to 6.0 are typical in substrates with pine bark and moss in the production of various species under nursery conditions [33].

On the other hand, the values that were reached in the electrical conductivity are also low in comparison with the recommended values; however, these values must not exceed 3.5 dS m^{-1} , since they could cause salinity problems [28].

4.2. Morphological Variables

4.2.1. Root Collar Diameter

The most widely used morphological attribute with which to estimate the quality of the seedlings is root collar diameter [8,11,34]. Because the survival and growth of plants in the field is associated with the root system of the seedling, the diameter has been defined as an important indicator [35,36]. In this sense, treatments with S4-M4 (30% peat + 30% bark + 40% sawdust + 4 g L⁻¹ of Multicote®) and S5-M2 (25% peat + 25% bark + 50% sawdust + 2 g L⁻¹ of Multicote®) stood out from the rest with respect to this variable; however, the criteria established by the NMX-AA-170-SCFI-2016 standard to produce *P. engelmannii*, establishes a root collar diameter of ≥ 5 mm [37]; that is, all treatments exceed this parameter.

For this variable, in *Pinus montezumae* Lamb. (species of similar growth), average values of 7.26 mm have been reported, and they were produced in substrate with composted pine sawdust (70%) + composted pine bark (15%) + vermiculite (15%) in combination with 4, 6 and 8 g L⁻¹ of Multicote® [17]. Also, in the same species, values of 9.7 to 11.4 mm are reported in substrates with different proportions of fresh pine sawdust (20% to 80%) in combination with peat moss (10% to 80%), composted pine bark (10%), perlite (10%), and vermiculite (10%) [21]. The information in the above indicates that the inclusion of sawdust in high proportions does promote suitable conditions for the seedlings to reach the appropriate diameter.

4.2.2. Dry Biomass (Aerial, Root, and Total)

In general, dry biomass was favoured in the treatments with the highest proportion of sawdust (40% to 70%), with addition of 4 and 6 g L⁻¹ of Multicote®; that is, with values higher than those found in the treatments that include the control substrate (S1-M2, S1-M4, and S1-M6). This can be attributed to the fact that the substrates with sawdust retained more moisture, a condition that could increase the availability of nutrients in these seedlings. This indicates that the fertilization scheme that was used (controlled-release fertilization plus foliar fertilization) was sufficient to prevent competition between the microorganisms and the seedlings, based on their dry biomass and diameter. Aguilera-Rodríguez et al. [17] reported similar values in aerial biomass (3.28–4.41), root biomass (0.91–1.11) and total biomass (4.19–5.51), when evaluating substrates with composted pine sawdust (70%) + composted pine bark (15%) + vermiculite (15%) in combination with 4, 6 and 8 g L⁻¹ of Multicote® in the production of *P. montezumae*.

In general, the root system of the seedlings produced in the different treatments, presented good physiognomy based on the points of growth observed in the root, possibly due to the ratio of the dry root biomass and the growth potential [8]. These results suggest a good performance of these seedlings in the field, since at the beginning of their establishment they will depend on the root system that was created in nursery [38], because the growth of the root is a determining factor in ensuring the development and establishment of the plantation [8].

4.3. Nutritional Analysis of the Foliage

When observing the nomograms of N, P and K, it is evident that with the dose of 2 g L⁻¹ of Multicote® an increase of biomass of needles is generated in most of the treatments that include fresh pine sawdust, but with effects of dilution of the elements, with reference to the treatment that only includes peat moss and composted bark (S1-M2). On the other hand, in the doses of 4 and 6 g L⁻¹ of Multicote®, it can be seen that the treatments containing sawdust are below their referents (S1-M4 and S1-M6) for the three elements. This decrease in the concentration of the elements may be due to the greater growth in aerial biomass, root biomass, and total biomass in plants grown on substrates that include sawdust, derived from their use in the production of photosynthates.

On the other hand, these concentrations of N, P and K are within the recommended levels of sufficiency for conifers (N 1.40%–2.20%, P 0.20%–0.40%, and K 0.40%–1.50%, respectively) [39],

in 17 treatments, and only in the case of N did the treatments S2-M2, S4-M2, S6-M2 and S7-M2 not reach the minimum value. However, in the ranges recommended for the species (N 1.1%–3.5%, P 0.1%–0.6% and K 0.2%–2.5%) [7], all treatments presented an acceptable value.

In *P. montezumae* produced in composite substrate with 70% compost pine sawdust + 15% compost pine bark + 15% vermiculite, with added high fertilization doses (8 g L⁻¹ of Multicote® and 8 g L⁻¹ of Osmocote Plus®), Aguilera-Rodríguez et al. [17] reported slightly lower values (0.79% to 1.33% in N, 0.06% to 1.16% in P, and 0.12% to 0.31% in K) compared to the values in the present study, despite using composted sawdust. It is possible that this was due to the fact that the controlled release fertilizers were not accompanied by foliar fertilization, or it is possible that this was because they had already been assimilated by the seedlings.

4.4. Costs

In this study, sawdust substrates were up to 67% cheaper than those substrates that include peat moss and bark, due to the low cost of sawdust, being that it is a locally-produced material. Otherwise, peat increases its cost due to increases in fuel consumption related to logistics, since the peat has to be imported from other countries, mainly from the U.S.A., hence it is important to replace or gradually reduce its use [40,41].

The main objective of the seedlings producers is to minimise the cost and to improve the quality of the seedlings [42]. Therefore, it is recommended that the managers of the forest nurseries use local materials in their culture media [41,43,44]. In addition, special attention should be paid to the use of simple and environmentally acceptable materials that are easily accessible to nursery producers [40,45]. Composted materials have disadvantages, because developing countries are not necessarily equipped with the appropriate infrastructure to treat and compost solid waste, which generates pollutants that negatively affect human health and that contaminate the water table [40]. For this reason, the inclusion of high proportions of fresh pine sawdust turns out to be a viable option and with favourable results in the morphology of *P. engelmannii*, likewise presenting no toxic waste or health risks during its production in the nursery.

5. Conclusions

The species *P. engelmannii* can be produced in a nursery in substrates with high proportions of fresh sawdust (up to 70%), with nutrient reserves (N, P and K) within the ranges recommended for the species. Based on the price of peat moss, composted bark and fresh sawdust, substrates with these materials can be 23% to 67% cheaper than the control substrate, depending on the proportion of sawdust in the mix. Finally, it is recommended to carry out tests to corroborate whether, after planting in the field, the same tendency of the best substrates and fertilization doses found in the nursery is maintained.

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