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Article

Raw sawdust substrates and fertilization in the plant quality of *Pinus cooperi* Blanco seedlings grown at the nursery

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Abstract:
In order to ensure the success of reforestation programs, it is necessary to use high quality seedlings. Using alternative substrates of wide availability and low cost (raw pine sawdust and composted pine bark) may be a viable option to produce such seedlings. Therefore, the effect of four substrates (composed of raw pine sawdust, composted pine bark and peat moss) in interaction with two controlled-release fertilizers (Multicote™ and Osmocote Plus™) on the quality of nursery-grown *Pinus cooperi* seedlings was assessed. The seedlings were planted in polystyrene trays with 77 cavities and a capacity of 170 mL per cavity. The substrates evaluated were: S1) 46 % peat moss + 54 % bark, S2) 30 % peat moss + 20 % bark + 50 % sawdust, S3) 25 % peat moss + 25 % bark + 50 % sawdust, and S4) 20 % peat moss + 30 % bark + 50 % sawdust —all of them combined with Multicote™ (18-06-12, N-P-K) and Osmocote Plus™ (15-09-12, N-P-K). The experimental design utilized was completely randomized, with a 4 × 2 factorial arrangement. In nine-month-old seedlings, the best results for the variables diameter, total biomass and Dickson quality index were found in substrate S1: 46 % peat moss + 54 % bark with 8 g L^{-1} of Multicote™. However, substrate S2: 30 % peat moss + 20 % bark + 50 % sawdust in combination with 8 g L^{-1} of Multicote™ also yielded acceptable values and, in addition, reduced the production costs by 39.8 %, due to the substrate and fertilizer.

Key words: Raw pine sawdust, plant quality, composted pine bark controlled-release fertilizer, peat moss, morphological variables.

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Introduction

196 million plants of the genus *Pinus* are produced in Mexico every year; this activity is important for the reforestation programs and commercial forest plantations. In the state of *Durango*, during the 2010–2015 period, the production of nursery-grown seedlings increased from 6.4 to 10 million seedlings per year (Conafor, 2016). In order for this vegetative material to survive and develop properly in field, it must have the adequate morphological and physiological characteristics; these are attained through a good management of the factors involved in their growth in the nursery: substrate, irrigation, fertilization, and pest and disease control, among others (Serrada, 2000; Prieto and Sáenz, 2011).

In forest nurseries, the substrate is a raw material of particular interest because its characteristics in terms of porosity, water retention, drainage and availability of nutrients are directly related to growth, to the production of dry matter and to the survival of the species (Sandoval *et al*., 2000; Zumkeller *et al*., 2009; Escobar and Buamscha, 2012). Normally, the substrates are formulated with balanced mixtures of organic and inorganic mater; these components are selected based on their stability, handling, root ball formation, health, availability and cost (Burés, 1999; Escobar, 2012).

The most widely used substrate in the country is a mixture of moss peat, perlite and vermiculite, in a 60:30:10 proportion, respectively. However, the high prices of peat moss (125 USD m^{-3}), perlite (114 USD m^{-3}) and vermiculite (151.5 USD m^{-3}), which are imported (Aguilera *et al*., 2016a), generate the need to seek other alternatives with regional materials that may replace or reduce their use (Tian *et al.*, 2017). Furthermore, the excessive extraction of peat moss causes important environmental damage to the ecosystems where it is collected (Aleandri *et al.*, 2015).

Certain options of substrates are based on waste of cattle (cow dung compost) and of the agro-food (coconut and coffee fibers) and forest (pine bark and sawdust) industries (Aguilera *et al.,* 2016a; Aguilera *et al.,* 2016b).

In Mexico, the use of raw pine sawdust is limited, prevailing only at the center of the country, with satisfactory results in the production of *Pinus pseudostrobus* Lindl. (Reyes *et al*., 2005; Aguilera *et al*., 2016a), *Pinus greggii* Engelm. (Maldonado *et al*., 2011), *Cedrela odorata* L. (Mateo *et al*., 2011) and *Pinus montezumae* Lamb. (Hernández-Zarate *et al*., 2014; Aguilera *et al*., 2016b). This has allowed growing seedlings with characteristics that agree with the morphological parameters established by the National Forestry Commission (*Comisión Nacional Forestal*, Conafor). Nevertheless, according to Buendía *et al.* (2016), the behavior of this material under different production conditions must be assessed.

In the state of *Durango*, the incorporation of raw pine sawdust as a component of the substrates is a viable option in the production of forest seedlings, as each year the forest industry generates approximately 461 777 m^3 of this material (Semarnat, 2015), which has a low cost but is little used (9.4 USD m^{-3}) (Fregoso *at al.,* 2017).

On the other hand, composted pine bark has been utilized as part of the substrates for approximately 10 years (Prieto *et al.*, 2009), due to its ample availability in the region and is cost (56.3 USD m^{-3}), which is lower than that of the materials cited above.

Because no material has by itself all the necessary characteristics for its use as a substrate, mixtures in which peat moss is an indispensable material are prepared; however, these require adding fertilizers in order to nourish the vegetative materials in propagation (Burés, 1999; Sánchez *et al.,* 2008). Fertilizers may be added directly to the substrate, in the form of controlled-release granulated products, which render their management easier, or by irrigation during the production process, and in some cases, they combine both alternatives. The type and amount of fertilizer must be carefully determined (Oliet *et al*., 1999; Dumroese *et al*., 2012), as various options may lead to different results in terms of profitability and of the quality of the plants.

Based on the above, the objectives of this study were: 1) to determine the physical and chemical characteristics of the substrates that were mixtures of raw pine sawdust, composted pine bark and peat moss; 2) to assess the influence of four substrates in combination with controlled-release fertilizers, on the growth and quality of nursery-grown *Pinus cooperi* seedlings, and 3) to determine the cost per plant, based on the substrates and fertilizers used. The hypotheses were that: 1) a substrate composed of raw pine sawdust in combination with peat moss and composted pine bark produces high-quality seedlings and reduces the nursery production costs, and 2) at least one controlled-release fertilizer enhances the quality of the seedlings.

Materials and Methods

Study area

The experiment was carried out at the "*General Francisco Villa*" forest nursery, located in the *ejido* known as *15 de Septiembre*, in *Durango*, *Dgo*., Mexico, at the coordinates 23°58'20.38" N and 104°35'55.83" W and an altitude of 1 875 masl. The study was performed in a baticenital greenhouse with zenithal, lateral and frontal ventilation, and an automated irrigation system with microsprinklers. The average minimum temperature was 7.8 °C; the average mean temperature, 18.8 °C, and the average maximum temperature, 34.4 °C.

Plant production and treatment

The cultivation cycle began in November 2014 and ended in July 2015; the seeds were collected in *San José Miravalles, San Dimas* municipality, *Durango*. Before planting, a pre-germination treatment, which consisted in soaking the seeds in water for 24 hours, and then disinfecting them during 5 minutes in a solution of 10 % commercial chlorine in 90 % water; *Captán*™ fungicide (N-trichloromethyl-4-ciylohexene-1,2-dicarboximide) was subsequently added, in doses of 2.5 g L^{-1}. The seeds were planted in polystyrene trays with 77 cavities, with a capacity of 170 mL per cavity. The substrates consisted of peat moss, composted bark of *Pinus douglasiana* Martínez and raw pine sawdust (with a particle size of 0.1 to 1.5 mm) obtained from sawn logs of *Pinus engelmannii* Carr., *Pinus cooperi* Blanco and *Pinus durangensis* Martínez.

Eight treatments derived from four substrates were assessed. The substrates were: S1) 46 % peat moss + 54 % composted pine bark (considered as the control because it was the substrate used at the greenhouse); S2) 30 % peat moss + 20 % composted pine bark + 50 % raw pine sawdust; S3) 25 % peat moss + 25 % composted pine bark + 50 % raw pine sawdust, and S4) 20 % peat moss + 30 % composted pine bark + 50 % raw pine sawdust. In addition, two controlled-release fertilizers were used: 1) Multicote™ 18N - $6P_2O_5$ - $12K_2O$ + 2MgO + micro-nutrients (Haifa Chemicals Ltd.) and 2) Osmocote Plus™ 15N - $9P_2O_5$ - 12 K_2O + micro-nutrients (eveRRIS ILC Fertilizer Company), both of which were applied at a fixed dose of 8 g L^{-1} and released nutrients through 8 to 9 months. During the cultivation cycle, the seedlings were irrigated with water only, *i.e.* no leaf fertilizers were added.

Physical and chemical characteristics of the substrates

The physical characteristics —aeration porosity (%), moisture retention porosity (%) and total porosity (%)— of all four substrates were determined using the method described by Landis (1990). As for their chemical characteristics, the pH measured in water and the electric conductivity (dS m^{-1}) were considered, based on the norm NOM-021-RECNAT-2000, in order to determine the fertility of the soils. The analyses were carried out at the *Laboratorio de Ciencias Ambientales del Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Durango, del Instituto Politécnico Nacional (IPN)* (Environmental Sciences Laboratory of the Interdisciplinary Research Center for Regional Integral Development, campus *Durango*, of the National Polytechnic Institute) (IPN).

Morphological variables

Six nine-month-old individuals per experimental unit were extracted; their height from the stem base to the apical bud (cm), with a Truper™ 14387 ruler; diameter at the stem base (mm) were measured with a SURTEK™ 122204 digital caliper; and their aerial, root and total dry biomass (g) was weighed. For the dry biomass, the seedlings were placed in paper bags and dehydrated in a FELISA™ FE-291D drying oven at 70 °C during 72 hours; they were subsequently weighed using an *Ohaus*™ PA214 analytical balance with a 0.0001 g precision.

The above variables were used to calculate the Dickson quality index (DQI) (Dickson *et al*., 1960):

$$DQI = \frac{TDW}{\left(\frac{ADW}{RDW} + \frac{H}{D}\right)}$$

Where:

TDW = Total dry weight

ADW = Dry weight of the aerial part

RDW = Root dry weight

H = Height of the plant

D = Diameter of the plant

Nitrogen, phosphorus and potassium concentrations

The nitrogen, phosphorus and potassium concentrations were determined based on representative samples of the foliage, consisting of needles from the middle part of each seedling (5 g per treatment), with three repetitions. The nitrogen content was estimated using the Kjeldahl method; the phosphorus content, using colorimetric analysis with phosphorus-vanadium molybdenum yellow complex, and the potassium content, by atom emission. All these analyses were performed at the *Laboratorio de Fertilidad de Suelos y Química Ambiental, del Colegio de Postgraduados, Montecillo, Estado de México* (Soil Fertility and Environmental Chemistry Laboratory of the College of Postgraduates in *Montecillo*, State of Mexico).

Cost of the substrate

The cost of the substrate was determined based on 170 mL of substrate per cavity, and the addition of 34 mL to the volume because of the compaction occurring when the cavities of the trays are filled. The value per liter of substrate was estimated in USD, being 0.088 for S1, 0.053, S2, 0.050 for S3 and 0.047 for S4. The cost of the fertilizers was estimated in USD, with values of 2.31 per kilogram of *Multicote*™ and 3.81 per kilogram of *Osmocote Plus*™.

Experimental design and statistical analysis

A totally random experimental design with a 4 × 2 factorial arrangement (four substrates and two fertilizers) was used. The experimental unit was made up of 77 seedlings (7 × 11) contained in polystyrene trays, with four repetitions per treatment. The following statistical model was used:

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk}$$

Where:

Y_{ijk} = Response obtained for the i^{th} of the A factor level and the j^{th} of the factor B level in the k^{th} repetition.

μ = Mean overall efect

A_i = Effect ascribed to the i^{th} level of factor A

B_j = Effect ascribed to the j^{th} level of factor B

$(AB)_{ij}$ = Effect ascribed to the interaction between the i^{th} level of factor A and the j^{th} level of factor B

e_{ijk} = Random error, where the e_{ijk} have a normal and independent distribution with a mean = 0 and a variance = σ^2

i = Number of levels of factor A (four substrates)

j = Number of levels of factor B (two fertilizers)

k = Number of repetitions (four)

The potential significant statistical differences between treatments were detected by means of a variance analysis using the GLM procedures; the variables with statistical significance were subjected to a Tukey mean comparison test ($P \leq 0.05$), using the statistical package SAS 9.0 (SAS, 2002).

Results and Discussion

Physical and chemical characteristics of the substrates

The aeration porosity varied by 1.5 % between treatments, with an interval of 31.4 to 32.7 %. The moisture retention porosity ranged between 32.0 % in S1 and 44.8 % in S2; *i.e.* it increased in those substrates that included sawdust, which caused a higher total porosity, with values ranging from 64.2 % in S1 to 76.2 % in S2 (Table 1).

Table 1. Physical and chemical characteristics of the substrates assessed in the production of *Pinus cooperi* Blanco.

Substrate	**Aeration porosity (%)**	**Moisture retention porosity (%)**	**Total porosity (%)**	**pH**	**Electric conductivity (dS m^{-1})**
S1(Control)	32.1	32.0	64.2	4.7	0.1
S2	31.4	44.8	76.2	4.7	0.1
S3	32.5	43.5	76.0	4.9	0.1
S4	32.7	41.8	74.5	5.0	0.1
RV	25 to 35	25 to 55	60 to 80	5 to 6.5	<1.0

S1 = 46 % peat moss + 54 % composted pine bark; S2 = 30 % peat moss + 20 % composted pine bark + 50 % raw pine sawdust; S3 = 25 % peat moss + 25 % composted pine bark + 50 % raw pine sawdust; S4 = 20 % peat moss + 30 % composted pine bark + 50 % raw pine sawdust. RV = Recommended values (Landis *et al.,* 1990; Mathers *et al.,* 2007).

The physical properties are relevant because they cannot be modified during the cultivation cycle; for this reason, the substrate should have the appropriate characteristics from the start (Cruz-Crespo *et al.,* 2013). Based on the production parameters of the nursery-grown conifer seedlings, the recommended interval for the aeration porosity is 25 to 35 % (Landis, 1990), which shows that a proper value was attained with all the treatments (Table 1). In this case, the particle size and the proportion of the materials in the substrates favored the availability of porous spaces (Cruz-Crespo *et al.*, 2013).

The assessed substrates with different proportions of peat moss, bark, sawdust, perlite and vermiculite (Hernández-Zarate *et al.*, 2004) exhibited similar values for aeration porosity in substrates with 40 % composted bark + 60 % sawdust and 60 % composted bark + 40 % sawdust; while for substrates with 80 % composted bark + 20 % sawdust and 60 % composted bark + 40 % sawdust, Sánchez *et al.* (2008) cite a value of 9 %, attributed to the fact that the utilized materials contained a large number of fine particles, which allowed for few air spaces.

With regard to moisture retention porosity, the recommended values are 25 to 55 % (Landis, 1990); in the present study, the lowest value (32.0 %) was for S1, while the substrates with sawdust increased due to the larger number of fine particles, with values ranging between 41.8 and 44.8 % (Table 1). Hernández-Zarate *et al.* (2014) obtained similar values (40 and 41 %) in substrates with 40 % composted bark + 60 % sawdust and 60 % composted bark + 40 % sawdust; in substrates with 70 and 80 % sawdust values of 63 to 65 % have been documented (Sánchez *et al*., 2008; Aguilera *et al.,* 2016b). Moisture retention increases in direct proportion to the percentage of sawdust, due to the absorption capacity of the latter; whereas bark has a low moisture retention capacity, as can be seen in S1 (which was 54 % bark); this can be corrected by mixing the bark with other materials with better moisture retention (García *et al.,* 2001; Cervantes *et al.,* 2018), such as sawdust and peat moss.

In the study here described, total porosity in all the substrates was within the recommended interval (60-80 %) (Landis, 1990), with values ranging from 64.2 to 75.6 % (Table 1). Again, the substrate with 54 % bark had the lowest records. The production of *Pinus montezumae* exhibited similar results (69 to 77 %) in the substrates composed of bark and sawdust (Hernández-Zarate *et al.*, 2014); this suggests that raw sawdust in combination with such materials as peat moss and pine bark produces substrates with a good balance in terms of these characteristics.

As for the assessed chemical characteristics, the average pH in substrates S1, S2 and S3 was 4.8, *i.e.* lightly more acid than in S4 (5.0); only S4 attained the minimum recommended value (pH = 5) (Table 1). However, the use of fertilizers and irrigation may produce a higher content of salts and cause the pH levels to increase by 0.5 to 1.0 units (Landis, 1990). Sánchez *et al.* (2008) (4.1 to 5.2) and Hernández-Zarate *et al.* (2014) cite similar values (4.3 to 4.7) for substrates with various combination of composted pine bark and sawdust, as well as a pH of 4.9 in the substrate containing 60 % raw sawdust + 20 % peat moss + 20 % composted pine bark (Castro *et al.*, 2018). Atland *et al.* (2014) point out that the typical values observed in the production of various nursery-grown species range between 4.0 and 6.0 when substrates with pine bark and peat moss are used.

In all substrates, the average value for electric conductivity was 0.1 dS m^{-1} (Table 1), while acceptable values range between 0.8 and 3.5 dS m^{-1}. When figures are above the 5.0 value, they indicate high salinity, as few seedlings can resist this condition. In this study, the substrates had values rated low (Mathers *et al.*, 2007). Aguilera *et al.* (2016b) also cite low values (0.04 dS m^{-1}) for a substrate with 70 % composted pine sawdust + 15 % composted pine bark + 15 % vermiculite; by incorporating 60 % raw sawdust + 20 % peat moss + 20 % composted pine bark; Castro *et al.* (2018) obtained 0.9 dS m^{-1}.

Morphological variables

The substrate and fertilizer factors did not produce evident significant differences in the height of the seedlings; however, there were statistically significant effects on the diameter, of 4.0 mm, both in S1 and in the substrate with Multicote^{TM}. As for the interaction of the assessed factors, there were significant differences in diameter; the best interaction resulted from S1 with Multicote^{TM}, with 4.0 mm, followed by substrates S2 and S3 combined with Multicote^{TM} (Table 2).

Table 2. Mean values, standard error and significances of the morphological variables assessed in *Pinus cooperi* Blanco*,* in response to the substrates and controlled-release fertilizers used during the November 2014 to July 2015 cultivation cycle.

Factor/Treatment	**Height (cm)**	**Diameter (mm)**	**Dry biomass (g)**			***Dickson* quality index**
			Aerial	**Root**	**Total**	
Substrate						
S1	15.1 ± 0.7 a	4.0 ± 0.1 a	2.1 ± 0.1 a	0.7 ± 0.0 a	2.8 ± 0.1 a	0.4 ± 0.0 a
S2	16.4 ± 0.7 a	3.8 ± 0.1 ab	1.8 ± 0.1 ab	0.7 ± 0.0 a	2.5 ± 0.2 ab	0.4 ± 0.0 ab
S3	16.2 ± 0.6 a	3.7 ± 0.1 ab	1.7 ± 0.1 ab	0.7 ± 0.0 a	2.4 ± 0.1 ab	0.4 ± 0.0 ab
S4	14.7 ± 0.7 a	3.6 ± 0.1 b	1.5 ± 0.1 b	0.6 ± 0.0 a	2.1 ± 0.1 b	0.3 ± 0.0 b
P	0.2074 ns	0.0147 *	0.0041 **	0.1509 ns	0.0096 **	0.0499 *
Fertilizer						
Multicote^{TM}	15.6 ± 0.5 a	4.0 ± 0.1 a	1.8 ± 0.1 a	0.7 ± 0.0 a	2.5 ± 0.1 a	0.4 ± 0.0 a
Osmocote Plus^{TM}	15.5 ± 0.5 a	3.6 ± 0.1 b	1.7 ± 0.1 a	0.6 ± 0.0 b	2.3 ± 0.1 a	0.3 ± 0.0 b
P	0.9298 ns	<0.0001 ***	0.4820 ns	0.0092 **	0.2133 ns	0.0043 **
Interaction						
S1- Multicote^{TM}	15.5 ± 1.0 a	4.2 ± 0.1 a	2.1 ± 0.2 a	0.8 ± 0.1 a	2.9 ± 0.2 a	0.5 ± 0.0 a
S2- Multicote^{TM}	16.6 ± 1.1 a	4.0 ± 0.1 ab	1.8 ± 0.2 ab	0.7 ± 0.0 ab	2.5 ± 0.2 ab	0.4 ± 0.0 ab
S3- Multicote^{TM}	16.3 ± 0.8 a	4.0 ± 0.1 ab	1.7 ± 0.1 ab	0.8 ± 0.0 ab	2.5 ± 0.2 ab	0.4 ± 0.0 ab
S4- Multicote^{TM}	13.0 ± 1.1 a	3.7 ± 0.1 abc	1.6 ± 0.1 ab	0.6 ± 0.0 ab	2.2 ± 0.2 ab	0.4 ± 0.0 ab
S1- Osmocote Plus^{TM}	14.6 ± 1.0 a	3.8 ± 0.1 abc	2.0 ± 0.2 ab	0.6 ± 0.1 ab	2.6 ± 0.2 ab	0.4 ± 0.0 ab
S2- Osmocote Plus^{TM}	16.2 ± 1.0 a	3.6 ± 0.1 bc	1.9 ± 0.2 ab	0.7 ± 0.1 ab	2.6 ± 0.2 ab	0.4 ± 0.0 ab
S3- Osmocote Plus^{TM}	16.0 ± 0.8 a	3.4 ± 0.1 c	1.6 ± 0.1 ab	0.6 ± 0.0 ab	2.2 ± 0.2 ab	0.3 ± 0.0 b
S4- Osmocote Plus^{TM}	15.4 ± 0.8 a	3.4 ± 0.1 c	1.5 ± 0.1 b	0.5 ± 0.0 b	2.0 ± 0.2 b	0.3 ± 0.0 b
P	0.5066 ns	<0.0001 ***	0.0353 *	0.0160 *	0.0359 *	0.0107 *

S1 = 46 % peat moss + 54 % composted pine bark; S2 = 30 % peat moss + 20 % composted pine bark + 50 % raw pine sawdust; S3 = 25 % peat moss + 25 % composted pine bark + 50 % raw pine sawdust; S4 = 20 % peat moss + 30 % composted pine bark + 50 % raw pine sawdust; Multicote^{TM} = 18N - $6P_2O_5$ - $12K_2O$ + 2MgO + micro nutrients; Osmocote Plus^{TM} = 15N - $9P_2O_5$ - 12 K_2O + micro nutrients. *P* = Limit probabilities in ANOVA; *= $p<0.05$; **= $p<0.01$; ***= $p<0.001$; ns = Non-significant. In each column, different letters for the same variable by factor indicate significant differences, according to Tukey ($P < 0.05$).

In regard to dry biomass, substrate had an effect on the production of aerial and total biomass; the highest values were found in S1 (2.1 g of aerial biomass and 2.8 g of total biomass); the fertilizer only influenced root biomass; notably, the use of Multicote^{TM} produced 0.7 g. The interaction between the factors was significant, particularly in S1 with Multicote^{TM} (Table 2).

Based on the NMX-AA-170-SCFI-2016 Mexican norm (Secretaría de Economía, 2016), the recommended height for *P. cooperi* is 15 to 20 cm, with a diameter of ≥ 4.0 mm; in this case, all other treatments, with the exception of S4, reached the minimum height. As for the diameter, the interactions of S1, S2 and S3 with Multicote^{TM} registered the minimum suggested value in the two variables; these measures may be attained with all the treatments, with an additional month of cultivation.

Aguilera *et al.* (2016a) produced ten-month-old *P. pseudostrobus* seedlings in a substrate with 60 % raw sawdust + 15 % composted pine bark + 15 % peat moss + 10 % vermiculite, in combination with high doses of controlled-release fertilizers (8 g L^{-1} Multicote^{TM}), with the following measures: a height of 23.3 cm, a diameter of 5.3 mm, a root dry weight of 1.3 g, and an aerial dry weight of 4.2 g.

Reyes *et al.* (2005) combined 80 % sawdust with 20 % earth, 20 % pine bark, 20 % peat moss or 20 % agrolyte, and added 5 g L^{-1} of Multicote^{TM} 18-6-12 in order to produce nine-month-old *P. pseudostrobus* seedlings with values below those cited above. Maldonado *et al.* (2011) grew nine-month-old *P. greggii* seedlings in substrates composed of 40, 60 or 80 % sawdust and 20, 40 or 60 % pine bark with

5 g L^{-1} Osmocote^{TM} 14-14-14 N-P-K, obtaining a smaller growth to that of the species mentioned before. In these two cases, the increase in the proportion of sawdust and the low doses of fertilizer did not favor an adequate growth of the seedlings, as, when organic matter decays in the sawdust, the microorganisms compete for the available nutrients.

The Dickson quality index revealed significant differences at the substrate level (with the highest result for S1) and fertilizer (notably Multicote^{TM}); in interaction, the combination of S1 and Multicote^{TM} proved statistically superior. The Dickson quality index relates the height and diameter data to the aerial/root biomass ratio. In this case, a higher index indicates better quality plants; *i.e*. these individuals have a more balanced aerial/root biomass ratio (Oliet, 2000). The highest value in the present study corresponded to S1 in combination with Multicote^{TM} (0.5) (Table 2).

The production of *P. pseudostrobus* seedlings in substrates that include high proportions of sawdust (60-80 %), Reyes *et al.* (2005) and Aguilera *et al.* (2016a) exhibited acceptable values for the DQI, which indicates that the inclusion of raw sawdust in the substrate produces high quality seedlings.

Nitrogen, phosphorus and potassium concentration

At the factor level, significant differences between substrates were registered only for nitrogen, notably in S1. Likewise, the differences in the interaction between the substrates and the fertilizers were significant only for this element, the highest concentration occurring in S1, with 8 g L^{-1} Osmocote Plus^{TM} (Table 3).

Table 3. Mean values, standard error and significances by substrate, fertilizer and interaction of nitrogen, phosphorus and potassium concentrations in the foliage of nine-month-old nursery-grown *Pinus cooperi* Blanco seedlings.

Factor/Treatment	**Nitrogen (%)**	**Phosphorus (%)**	**Potassium (%)**
Substrate			
S1	1.3 ± 0.1 a	0.2 ± 0.0 a	1.0 ± 0.1 a
S2	1.0 ± 0.0 b	0.2 ± 0.0 a	1.0 ± 0.0 a
S3	1.0 ± 0.0 b	0.2 ± 0.0 a	1.0 ± 0.0 a
S4	0.9 ± 0.0 b	0.2 ± 0.0 a	0.9 ± 0.1 a
P	<.0001 ***	0.2697 ns	0.7396 ns
Fertilizer			
Multicote™	1.0 ± 0.0 a	0.2 ± 0.0 a	1.0 ± 0.0 a
Osmocote Plus™	1.1 ± 0.1 a	0.2 ± 0.0 a	0.9 ± 0.1 a
P	0.6283 ns	0.6283 ns	0.3241 ns
Interaction			
S1- Multicote™	1.2 ± 0.0 ab	0.2 ± 0.0 a	1.0 ± 0.1 a
S2- Multicote™	1.1 ± 0.1 bc	0.2 ± 0.0 a	0.9 ± 0.0 a
S3- Multicote™	1.0 ± 0.0 bc	0.2 ± 0.0 a	1.0 ± 0.1 a
S4- Multicote™	0.9 ± 0.0 c	0.2 ± 0.0 a	1.0 ± 0.1 a
S1- Osmocote Plus™	1.4 ± 0.0 a	0.2 ± 0.0 a	0.9 ± 0.1 a
S2- Osmocote Plus™	1.0 ± 0.0 bc	0.2 ± 0.0 a	1.0 ± 0.1 a
S3- Osmocote Plus™	1.0 ± 0.1 bc	0.2 ± 0.0 a	1.0 ± 0.0 a
S4- Osmocote Plus™	0.9 ± 0.0 bc	0.2 ± 0.0 a	0.9 ± 0.1 a
P	<.0001 ***	0.5024 ns	0.8977 ns

RV	1.3-3.5	0.2-0.6	0.7-2.5

S1 = 46 % peat moss + 54 % composted pine bark; S2 = 30 % peat moss + 20 % composted pine bark + 50 % raw pine sawdust; S3 = 25 % peat moss + 25 % composted pine bark + 50 % raw pine sawdust; S4 = 20 % peat moss + 30 % composted pine bark + 50 % raw pine sawdust; Multicote™ = $18N - 6P_2O_5 - 12K_2O + 2MgO$ + micro nutrients; Osmocote Plus™ = $15N - 9P_2O_5 - 12K_2O$ + micro nutrients. *P* = Limit probabilities in ANOVA. * = p<0.01; *** = p< 0.001; ns = Non significant. In each column, different letters for the same variable by factor indicate significant differences, according to Tukey ($P < 0.05$); RV = Recommended values (Prieto and Sáenz, 2011).

Based on the intervals suggested by Prieto and Sáenz (2011), the nitrogen values are good in the interaction of S1 with Osmocote Plus™, and so are the concentrations of phosphorus and potassium in all the treatments (Table 3).

Pinus montezumae produced in a substrate with 70 % composted pine sawdust + 15 % composted pine bark + 15 % vermiculite and high doses of fertilizers (8 g L^{-1} of Multicote™ and 8 g L^{-1} of Osmocote Plus™) exhibited similar N, P and K values to those found in the present study (Aguilera *et al.,* 2016b), although 20 % less sawdust was used, without composting, in the assay documented herein; this indicates that adding high doses of fertilizer with sawdust as a component of the substrate favors the growth of seedlings, as the nutritional demand of the microbioligical activity and the development of the plant is met by these doses. Furthermore, controlled-release fertilizers do not immediately release 100 % of the nutrients, as their role is to do so gradually, according to the level of development of the plant; this prevents losses from leaching, resulting in maximum efficiency of both the fertilizer and the plant (Rose *et al.*, 2004).

Cost of the substrates

According to commercial estimates, substrates S2, S3 and S4, which contain raw sawdust, are 39.8, 43.2 and 46.6 % cheaper, respectively, than S1. Substrates with Multicote™ were 21.6 % cheaper than those with Osmocote™. For this reason, the cheapest interactions are S2, S3 and S4, in combination with 8 g L^{-1} of Multicote™ (Table 4). The difference in cost in the substrates and the inclusion of sawdust, which is a low-cost material, is due to the reduction in the proportion of peat moss, an expensive material.

Table 4. Cost of the substrates with fertilizer per *Pinus cooperi* Blanco plant, produced in polystyrene trays with 77 cavities of 170 mL each.

Factor/Treatment	**Cost per plant (USD)**
Substrate	
S1	0.0179
S2	0.0108
S3	0.0102
S4	0.0095
Fertilizer	
Multicote™	0.0038
Osmocote Plus™	0.0062
Interaction	
S1- Multicote™	0.0217
S2- Multicote™	0.0146
S3- Multicote™	0.0140

S4- Multicote^{TM}	0.0133
S1- Osmocote Plus^{TM}	0.0241
S2- Osmocote Plus^{TM}	0.0170
S3- Osmocote Plus^{TM}	0.0164
S4- Osmocote Plus^{TM}	0.0157

S1 = 46 % peat moss + 54 % composted pine bark; S2 = 30 % peat moss + 20 % composted pine bark + 50 % raw pine sawdust; S3 = 25 % peat moss + 25 % composted pine bark + 50 % raw pine sawdust; S4 = 20 % peat moss + 30 % composted pine bark + 50 % raw pine sawdust; Multicote^{TM} = 18N - 6P_2O_5 - 12K_2O + 2MgO + micro nutrients; Osmocote Plus^{TM} = 15N - 9P_2O_5 - 12 K_2O + micro nutrients.

According to Escobar and Buamscha (2012), the best substrate will be the one that is available nearby the nursery and has the lowest price. In terms of these characteristics, S2 makes it possible to produce seedlings with acceptable characteristics for in field establishment, without health problems and with 39.8 % savings; therefore, it is a substrate with a potential for the production of the studied species.

Conclusions

Substrates containing raw pine sawdust have acceptable physical and chemical characteristics for the production of forest species, notably the treatment using substrate S2 —30 % peat moss + 20 % composted pine bark + 50 % raw pine sawdust, combined with 8 g L^{-1} of Multicote^{TM}—, which allows the production of seedlings with adequate characteristics of height, diameter, total biomass and Dickson quality index; furthermore, this treatment reduces the production costs by 39.8 %.

Based on these results, the raw pine sawdust included in the substrate is a viable alternative, with a lower cost, to produce nursery-grown *Pinus cooperi* seedlings.

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Conflict of interests

The authors declare no conflict of interests.

Contribution by author

María Mónica González Orozco: establishment of the experiment, collection, capture and statistical analysis of the data and drafting of the document; José Ángel Prieto Ruíz: design and establishment of the experiment, review and editing of the document; Arnulfo Aldrete: counselling on the treatments to be evaluated and experimental design to be used, as well as review of the document; José Ciro Hernández Díaz: counselling on the results of the analysis of costs and review of the document; Jorge Armando Chávez Simental: review of the document; Rodrigo Rodríguez Laguna: review of the document.

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