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Research article

Supervivencia de injertos de tres especies de *Pinus* con dos técnicas y dos métodos cicatrizantes

Survival of grafts of three *Pinus* species with two techniques and two healing methods

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Abstract

Grafting is the most widely used vegetative propagation method in conifers to clone superior genotypes of forest species. The objective of the present study was to evaluate the effect of two grafting techniques (terminal cleavage and side veneer) and two healing methods (natural and application of healing wax) in three economically important species in *Durango*: *Pinus engelmannii*, *P. cooperi*, and *P. durangensis*. The experiment was established using a randomized complete block design with a 3×2×2 factorial arrangement and nine replications. Final survival was assessed at 180 days. The effect of individual treatments and their interactions was determined with Kruskal-Wallis, Dunn, and Dunnett tests. Significant differences (p<0.05) were obtained; the highest survival rate for *P. engelmannii* was 32.9 %, 16.3 % with side-veneer grafting, and 16.9 % with the use of healing wax. The double interactions between species and grafting technique, and between species and healing method showed significant differences (p<0.05), in addition to the triple interaction. The highest survival values were observed in side-veneer and terminal-cleft grafts of *P. engelmannii*, with 40 and 25.8 %, respectively, as well as in the grafts of *P. engelmannii* with wax application, whose value was 43.3 %. The best triple interaction was that of side-veneer grafts of *P. engelmannii* with healing wax (50 %). *P. engelmannii* responded best to grafting. For *P. cooperi* and *P. durangensis*, it is recommended to search for alternative grafting techniques.

Key words: Healing wax for grafting, cloning of superior genotypes, grafting of pines, side-veneer grafting, terminal-cleft grafting, vegetative propagation.

Resumen

El injertado es el método de propagación vegetativa más utilizado en coníferas para clonar genotipos superiores de especies forestales. El objetivo del presente estudio fue evaluar el efecto de dos técnicas de injertado (fisura terminal y enchapado lateral) y dos métodos de cicatrización (natural y aplicación de cera cicatrizante) en tres especies de importancia económica en Durango: *Pinus engelmannii*, *P. cooperi* y *P. durangensis*. El experimento se estableció con un diseño en bloques completos al azar con arreglo factorial 3×2×2 y nueve repeticiones. La supervivencia final se evaluó a los 180 días. El efecto de los tratamientos individuales y sus interacciones se determinó con las pruebas *Kruskal-Wallis*, *Dunn* y *Dunnett*. Se obtuvieron diferencias significativas (p<0.05); la supervivencia mayor fue de 32.9 % en *P. engelmannii*, 16.3 % en enchapado lateral y 16.9 % con cera cicatrizante. Las dobles interacciones especie × técnica de injerto y especie × método de cicatrización registraron diferencias significativas (p<0.05), además de la triple interacción. Los valores más altos de supervivencia se observaron en injertos de enchapado lateral y fisura terminal de *P. engelmannii*, con 40 y 25.8 %, respectivamente, así como en los injertos de *P. engelmannii* con aplicación de cera, cuyo valor fue de 43.3 %. La mejor triple interacción fue la de injertos de enchapado lateral de *P. engelmannii* con cera cicatrizante (50 %). *P. engelmannii* respondió mejor al injertado. En *P. cooperi* y *P. durangensis* se recomienda buscar otras técnicas de injertado.

Palabras clave: Cera cicatrizante para injertos, clonación de genotipos superiores, injertado de pinos, injerto de enchapado lateral, injerto de fisura terminal, propagación vegetativa.

Introduction

Genetic improvement is central to the sustainable management of forest resources (Zobel and Talbert, 1984), as it allows the obtainment seeds of high genetic quality and abundant quantity (Yuan *et al*., 2016) from sexual or asexual seed orchards —in the latter, with greater genetic gain, where controlled pollination can be carried out to cross individuals of high genetic value (Stewart *et al*., 2016).

In order to establish asexual seed orchards, trees with phenotypic characteristics of economic interest are cloned; the most common method used is grafting (Muñoz *et al*., 2013). Grafting techniques —mainly side veneer and terminal cleft— have been evaluated in the propagation of Mexican pines such as *Pinus patula* Schltdl. & Cham. (Aparicio-Rentería *et al*., 2013; González-Jiménez *et al*., 2022), *P. pseudostrobus* var. *oaxacana* (Mirov) S. G. Harrison (Barrera-Ramírez *et al*., 2021), *P. engelmannii* Carrière (Pérez-Luna *et al*., 2021), and *P. rzedowskii* Madrigal & Caball. Del. (Solorio-Barragán *et al*., 2021), with different results for each.

However, since in most of the grafting studies of *Pinus* species the survival rate has been less than 50 %, it is necessary to look for alternative techniques to increase this percentage (Pérez-Luna *et al*., 2020a) in which the age and quality of the rootstock plant are considered as important factors (Castro-Garibay *et al*., 2022; González-Jiménez *et al*., 2022;). As for scions, the use of shoots from the last year of growth favors grafting (Pérez-Luna *et al*., 2021). On the other hand, protecting the healing area with waxes or pastes strengthens the graft union and reduces the risk of tissue rotting (Muñoz *et al*., 2013); however, this subject has been little studied (Pérez-Luna *et al*., 2020a).

Pinus engelmannii, *P*. *cooperi* C. E. Blanco, and *P*. *durangensis* Martínez are species of the Ponderosae subsection distributed in northern Mexico and the southern United States of America, in the *Sierra Madre Occidental* (García and González, 2003); these are also widely used for establishing commercial forestry plantations in northern Mexico, particularly in the state of *Durango* (Martínez and Prieto, 2011).

Therefore, the objective of this study was to evaluate the effect of the grafting technique and the healing method on the survival of grafts of *P*. *engelmannii*, *P*. *cooperi*, and *P*. *durangensis*. The hypothesis was that there is a significant effect on graft survival in terms of species, grafting technique, and healing method.

Materials and Methods

Scion collection

40 scions per orteto were collected from 10 superior trees of each evaluated species. Superior trees were selected based on their outstanding phenotypic characteristics: dominant height, straight stem, efficient natural pruning, small crown size, branch insertion at an angle close to 90° and no damage by pests or diseases (Pérez-Luna *et al*., 2020a). *P*. *engelmannii* scions were collected in a seed stand located in *La Campana ejido*, municipality of *Pueblo Nuevo*, *Durango*, located at coordinates 23°48’51.2’’ N and 105°29’55.4’’ W, at an altitude of 2 802 m. *P*. *cooperi* and *P*. *durangensis* scions were obtained from a seed stand in *La Victoria ejido*, *Pueblo Nuevo* municipality, state of *Durango*, located at coordinates 23°45’12.13’’ N and 105°22’15.74’’ W, at an altitude of 2 653 m above sea level.

Collections were carried out between January 26th and 30th, 2014; for transfer to the grafting site, the plant material was placed in plastic boxes covered with sawdust moistened with a water-based solution containing 5 g L^{-1} of Captan fungicide (Captan™ 50 WP). The scions were grafted one day after harvesting.

Grafting site

Grafting was carried out in a 20 m long × 5 m wide (100 m^2) and 2.4 m high greenhouse, with a white plastic cover 720-micron caliber, located at the *Guadiana* Valley Experimental Field of the *Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias* (*INIFAP*) in *Durango*, Mexico, at the coordinates 23°59’11.14’’ N and 104°37’20.54’’ W, at an altitude of 1 879 masl. A 60 % light retention shade net was placed over the plastic cover to reduce the incidence of solar radiation inside the greenhouse. Temperature (Figure 1a) and relative humidity (Figure 1b) were registered during the months of the experiment with an Elitech® model Rc-4hc Data Logger thermohygrometer.

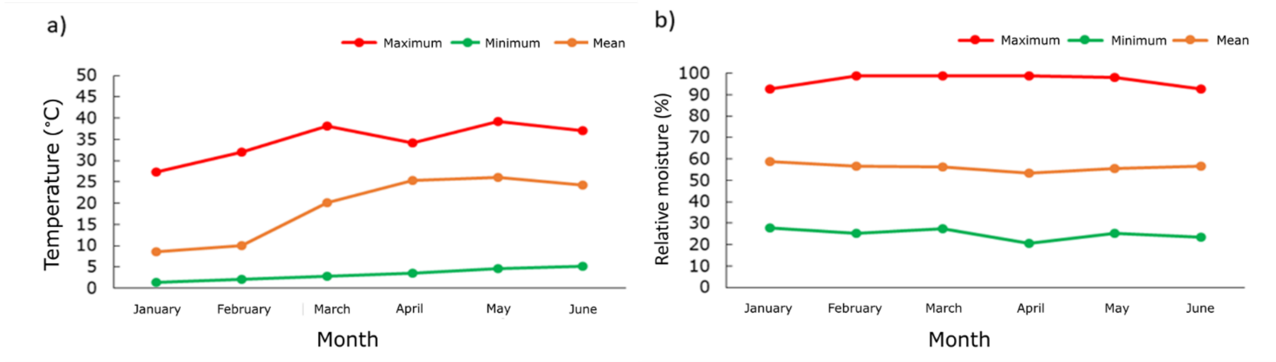


Figure 1. Monthly record of maximum, minimum, and average values of: a) temperature, and b) relative humidity.

Rootstock production

The rootstock plant was produced in 77-cavity expanded polystyrene trays with a capacity of 170 mL per cavity, with a peat-based substrate (60 %), perlite (20 %), and vermiculite (20 %). At eight months of age, plants were repotted into 5 L, 700 *μ*-gauge black plastic bags, with a substrate composed of peat (60 %) and composted bark (40 %). Rootstock patterns were three years old at the time of the grafting. Table 1 shows the average height and diameter characteristics of rootstocks, as well as the scion diameter and grafting height by species.

Table 1. Means ± standard deviation of the dasometric measurements of rootstocks and grafted scions.

Species	Rootstock plant height (cm)	Rootstock plant diameter (mm)	Scion diameter (mm)	Grafting height (cm)
Pinus engelmannii Carrière	48.13±10.98	13.73±2.39	13.53±2.42	30.26±11.07
Pinus cooperi C. E. Blanco	50.91±16.16	8.58±1.95	8.35±1.85	33.10±16.17
Pinus durangensis Martínez	54.59±10.64	9.05±1.60	8.74±1.57	26.33±8.57

Grafting

Grafting was performed on January 27th and 31st, 2014, using scions with dormant buds, the phenological stage recommended for conifer grafting (Muñoz *et al*., 2013). The terminal-cleft graft was made by cutting the rootstock stem at the height where its diameter coincided with that of the scion (Table 1), followed by a radial cut with a depth of 4 cm in the middle part of the cross section of the rootstock (Figure 2a). Two tangential cuts of 4 cm in length were made on the scion at the ends of the twig (Figure 2b), so that each cut of the scion ended with a slight inclination at the bottom to form a wedge that was placed and tied with the cut made in the rootstock plant (Figure 2c).

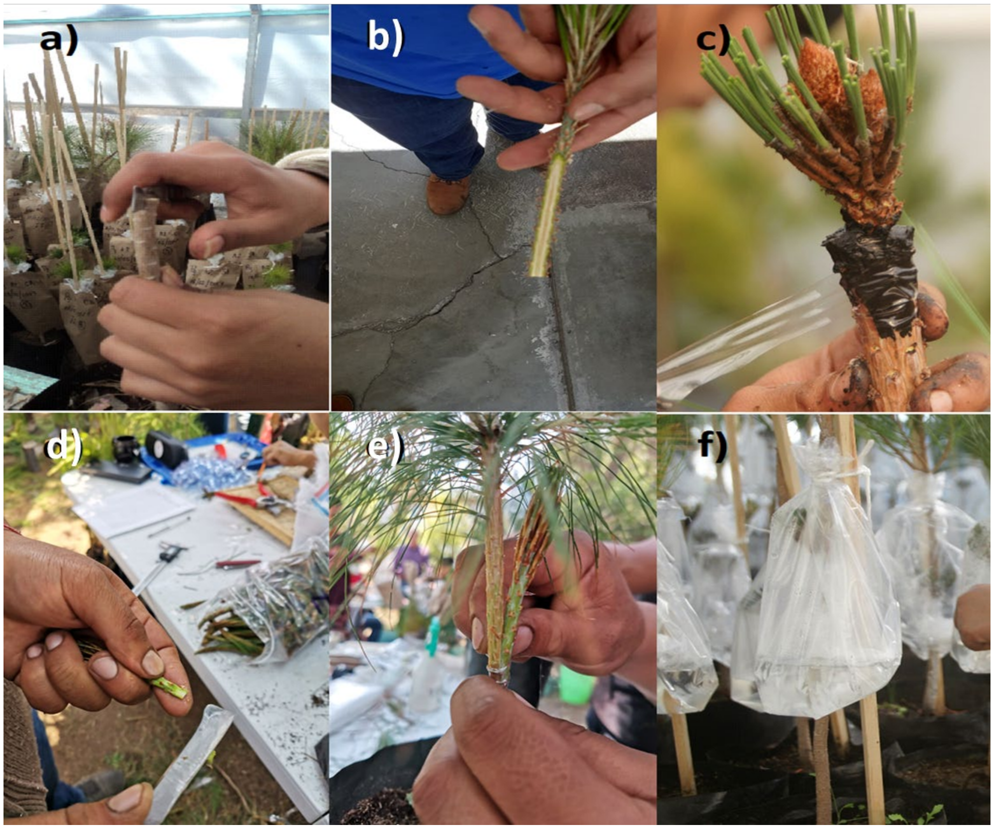


Figure 1. Grafting techniques: a) Transversal and longitudinal cut made to the rootstock plant; b) Cut in the scion for terminal-cleft grafting; c) Terminal cleft-graft joint with application of healing wax in its external section; d) Scion cutting for side veneer grafting; e) Side-veneer graft joint; f) Microclimate generated to maintain high relative humidity in the grafting area.

For the side-veneer graft, a 4 cm long tangential cut was made where the diameter of the scion and the rootstock coincided, with a small 1 cm deep slit at the base of the cut (Figure 2d). A tangential cut of 5 cm in length was made in one of the scion veneers, and a second tangential cut was made in the veneer parallel to the base of the first cut, 1 cm long, to achieve a proper match with the slit-shaped cuts generated in the rootstock plant (Figure 2e). The grafts were performed by a single grafter.

The graft joint was tied with 4 mm glass rubber and sealed with white vinyl paint. In order to reduce the risk of pathogen contamination in the graft area, the paint was treated with Captan fungicide (Captan® 50 WP) at a dose of 5 g L^{-1}. The high relative humidity in the grafting area was kept high with a transparent plastic bag containing water (microclimate) (Figure 2f). On top of the microclimate bag, a brown paper bag was placed to improve the temperature conditions and prevent direct solar radiation from affecting the graft. The rootstocks of the grafts were irrigated every other day with water and water-soluble triple 19 fertilizer (Poly feed GG®) at a dose of 3 g L^{-1}. The brown paper bag was removed 30 days after grafting; then, at 45 days, an opening was made in the bags containing the water, and at 60 days the water was removed from the microclimate. Finally, after 70 days, the microclimate was completely eliminated.

Treatments and experimental design

Twelve treatments were evaluated, being the product of three species (*P. engelmannii*, *P. cooperi*, and *P. durangensis*), two grafting techniques (terminal cleft and side veneer) and two healing methods (natural and application of Arbolsán® healing wax). By species, 288 grafts were performed: 144 with the lateral plating technique and 144 with the terminal cleft technique. Of each technique, half (72) were treated with resin-based healing wax (Arbolsán®) in the external section of the graft joint, without obstructing the cambium of the scion and of the rootstock plant; the other half (72) were not protected with wax. A total of 864 grafts were performed.

The treatments were distributed in a randomized complete block experimental design with a 3×2×2 factorial arrangements. Each block contained the individual factors evaluated (species, grafting technique, and healing method), and 96 grafts

—48 terminal-cleft and 48 side-veneer grafts— were evaluated. For each technique, 16 grafts were performed for each evaluated species; eight grafts were carried out with natural healing, and eight grafts, with healing paste per taxon. One block had nine replicates, with eight grafts per experimental unit. The response variable was survival, and it was evaluated at 60, 120, and 180 days.

The statistical model used was:

$$Y_{ijklm} = \mu + \beta_i + A_j + B_k + C_l + AB_{jk} + AC_{jl} + ABC_{jkl} + \epsilon_{ijklm}$$

Where:

Y_{ijklm} = Value of the response variable of the m^{th} repetition of the i^{th} block, j^{th} level of A, k^{th} level of B, and m^{th} level of C

μ = Overall average

A_j = Effect of the j^{th} level of factor A (species)

B_k = Effect of the k^{th} level of factor B (grafting technique)

C_l = Effect of the l^{th} level of factor C (healing method)

AB_{jk} = Effect of the interaction of the j^{th} level of A and k^{th} level of B

AC_{jl} = Effect of the interaction of the j^{th} level of A and l^{th} level of C

ABC_{jkl} = Effect of the interaction of the j^{th} level of A, k^{th} level of B, and l^{th} level of C

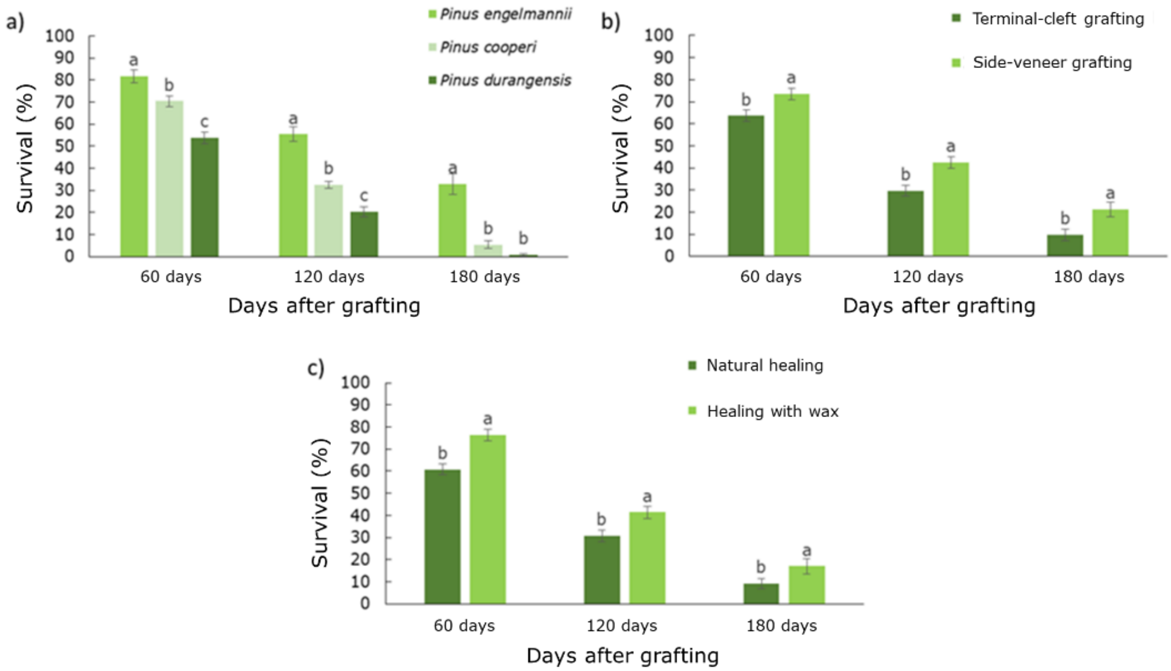
ϵ_{ijklm} = Experimental error

Statistical analysis

The normality of the data was tested using the Shapiro-Wilk and Kolmogorov-Smirnov tests. In order to evaluate the effect of individual treatments (species, grafting technique, and healing method) on graft survival, the Kruskal Wallis of medians and Dunn’s test were applied. Furthermore, Dunnett's median test was performed to determine the effect of double interactions between treatments and their triple interaction. The statistical significance value used in all analyses was $p<0.05$. Analyses were carried out with R® statistical software version 3.5.3 (R Core Team, 2020).

Results and Discussion

Significant differences in graft survival between the study species were obtained at 60 days ($p=0.003$), 120 days ($p=0.0002$) and 180 days ($p=2.7\times10^{-13}$). *P. engelmannii* grafts represented the first statistical group with 81.6, 55.4, and 32.9 % at 60, 120, and 180 days after grafting, respectively. At 180 days, *P. cooperi* and *P. durangensis* had 5.4 % and 0.8 % survival, respectively (Figure 3a).



Different letters indicate significant statistical differences between treatments according to Dunn's test. The bars represent the standard error.

Figure 3. Graft survival of *Pinus engelmannii*, *P. durangensis* and *P. cooperi*: a) by effect of grafting technique, b) by effect of species, and c) by effect of healing method.

As for graft survival, significant statistical differences by technique effect were observed between the study species at 60 days (p=0.043), 120 days (p=0.04), and 180 days (p=0.036). For both techniques, there was a drastic increase in mortality between 60 and 120 days post-grafting, as survival with both the terminal-cleft and side-veneer techniques decreased from 63.3 to 29.7 % and from 73.6 to 42.5 %, respectively. At 180 days, the highest survival rate was obtained with the side-veneer technique with 21.3 %, while with the terminal-cleft technique, the rate was 9.7 % (Figure 3b).

Significant statistical differences were also registered in the effect of the healing method at 60 days (p=0.011), 120 days (p=0.03) and 180 days (p=0.013); although there was high mortality between evaluations in both healing methods, the application of healing wax favored graft survival (76.3, 41.3 and 16.9 %, at 60, 120 and 180 days, respectively), compared to natural healing (without wax), with which the rates obtained at the same times were 60.8, 30.8 and 9.1 % (Figure 3c).

Several studies indicate that the best grafting technique for propagating species of the *Pinus* genus is the end-grafting technique. With this technique, Almqvist (2013) observed 84.7 % survival in *P. sylvestris* L., and Pérez-Luna *et al*. (2021), 80 % in *P. engelmannii*. Likewise, Castro-Garibay *et al*. (2022) cited 89 % survival rate in *P. greggii* var. *australis* Donahue, Jeffrey K. & López A., R. While, with the lateral plating technique, survival in *P. rzedowskii* was above 80 % (Solorio-Barragán *et al*., 2021), and 73 % in *P*. *patula* (González-Jiménez *et al*., 2022). On the other hand, Aparicio-Rentería *et al*. (2013) and Barrera-Ramírez *et al*. (2021) estimated 29 and 30 % survival rates in *P*. *patula* and *P*. *pseudostrobus* var. *oaxacana*, respectively.

In this study, survival rates with both techniques were low: below 10 % at 180 days in terminal cleft grafts, and 21.3 % with side veneer. The above may be attributed to factors unrelated to the object of evaluation in the research; one of these factors is the age of the rootstock plant, given that authors such as Castro-Garibay *et al*. (2022) and González-Jiménez *et al*. (2022) recommend the use of rootstock plants no older than one year of age. However, in certain slow-growing species, it is complicated to use rootstocks of that age, because by that time these do not reach the same growth in diameter as the bud to be grafted. Particularly *P*. *engelmannii*, which exhibits a cespitose state (Pérez-Luna *et al*., 2019), and for which three-year-old rootstocks are required. On the other hand, Pérez-Luna *et al.* (2020b) suggest keeping the grafts at temperatures no higher than 30 °C, which was exceeded in the present study (Figure 1a).

The large difference in survival in *P. engelmannii*, compared to *P. cooperi* and *P. durangensis* is indicative of the greater suitability of *P. engelmannii* for propagation by grafting. In this regard, Hibbert-Frey *et al.* (2011) note that not all conifer species can be successfully cloned by grafting. Also, it is documented that there are species that are susceptible to better propagation by rooting cuttings, such as *P. leiophylla* Schiede ex Schltdl. & Cham. (Cuevas, 2014) and *Abies fraseri* (Pursh) Poir. (Rosier *et al*., 2005).

On the other hand, the effect of healing agents has been little studied in conifer species. White *et al*. (1983) and McKeand *et al*. (1987) obtained 83 and 85 % survival in *Pinus taeda* L. by applying paraffin and resin-based wax at the graft junction, respectively. Blada and Panea (2011) in *Picea pungens* var. *glauca* Regel registered 41 and 87 % survival rates in paraffin and eco-wax grafts, respectively.

Regarding the effect of the interactions, significant differences ($p<0.05$) were observed in the interaction between species and the grafting technique factor in the three evaluations, with different levels of success, achieving more favorable results in *P. engelmannii* with both grafting techniques and a final survival rate of 40 and 25 % in side-veneer and terminal-cleft grafts, respectively. The second group included the four interactions with the two techniques (terminal cleft and side veneer) for the species *P*. *cooperi* and *P*. *durangensis* (Table 2).

Table 2. Graft survival rates due to the effect of the double interactions between the various levels of the evaluated treatments.

Double grafting technique x species interaction				
		Mean survival (%)± *SE*		
Species	**GT**	Days after grafting		
		60 (p=0.002)	120 (p=0.002)	180 (p=0.004)
Pe	TC	75.0±4.6 a	45.0±4.0 b	25.8±5.9 a
Pc		67.5±3.3 b	32.5±2.5 c	3.3±1.9 b
Pd		48.3±3.3 c	11.6±2.7 d	0.0±0.0 b
Pe	SV	88.3±3.4 a	65.8±4.0 a	40.0±6.8 a
Pc		73.3±3.7 a	32.5±2.2 c	7.5±2.8 b
Pd		59.1±4.1 b	29.1±2.3 c	1.6±1.1 b
Double interaction between species and method of healing				
Species	**MH**	Mean survival (%)± *SE*		

		Days after grafting		
		60 (p=0.008)	120 (p=0.003)	180 (p=0.0003)
Pe	NH	72.5±4.5 b	47.5±4.7 b	22.5±5.3 b
	HW	90.8±2.8 a	63.3±3.9 a	43.3±6.9 a
Pc	NH	64.1±2.5 c	28.3±2.4 d	4.1±2.3 c
	HW	76.6±3.8 b	36.6±1.9 c	6.6±2.5 bc
Pd	NH	45.8±3.6 d	16.6±2.9 d	0.8±0.8 c
	HW	61.6±3.4 c	24.1±3.3 d	0.8±0.8 c

Double interaction between grafting technique and method of healing

GT	**MH**	Mean survival (%)± *SE*		
		Days after grafting		
		60 (p=0.04)	120 (p=0.04)	180 (p=0.68)
TC	NH	53.8±3.1 b	23.3±2.8 b	5.0±2.2 a
	HW	73.3±3.3 a	36.1±3.9 ab	14.4±4.4 a
SV	NH	67.7±3.5 ab	38.3±3.9 a	13.3±3.8 a
	HW	79.4±3.6 a	46.6±3.7 a	19.4±5.3 a

GT = Grafting technique; MH = Method of healing; *SE* = Standard error; TC = Terminal cleft; SV = Side veneer; Pe = *Pinus engelmannii* Carrière; Pc = *Pinus cooperi* C. E. Blanco; Pd = *Pinus durangensis* Martínez; NH = Natural healing; HW = Healing with wax. Different letters indicate significant statistical differences between interactions according to Dunnett's median comparison test.

In the interaction between the species and the method of healing, significant differences (p<0.05) were obtained in the three evaluations; the interactions were divided into four statistical groups. The largest group corresponded to *P. engelmannii* grafts with healing wax, with a final survival rate of 43.3 %. Statistical differences (p<0.05) were only detected at 60 and 120 days in the interaction between the grafting technique and the method of healing, with the best results at

60 days, when healing wax was applied, and at 120 days with the side-veneer technique, regardless of the type of protection (Table 2).

Chávez (2016) evaluated terminal-cleft grafting of *P. engelmannii*, *P. cooperi* and *P. durangensis*, and cited 20, 34, and 0 % survival, respectively, three months after grafting; the result obtained in this work confirms how difficult it is to propagate the species evaluated, at least with the methodology employed.

The triple interaction exhibited significant statistical differences ($p<0.05$) in all evaluations. The most favorable interaction in the three evaluations occurred in *P. engelmannii* at 60 days with the use of healing wax, with both grafting techniques, at 120 days only the dominance of the species prevailed, and at 180 days, the side-veneer graft with application of healing wax stood out. The results with lower survival corresponded to *P. cooperi* and *P. durangensis*, regardless of the factor, which indicates that not all species respond in the same way to grafting (Table 3).

Table 3. Graft survival due to the effect of the triple interaction between the different levels of the evaluated treatments.

Species	**GT**	**MH**	**Mean survival (%)± *SE***		
			Days after grafting		
			60 (p=0.010)	120 (p=0.006)	180 (p=0.004)
Pe	TC	NH	63.3±6.4 c	31.6±2.9 b	15.0±5.8 bcd
		HW	86.6±4.1 a	58.3±4.4 a	36.6±9.5 ab
Pc		NH	58.3±2.7 c	30.0±4.1 b	0.0±0.0 d
		HW	76.6±4.4 b	35.0±2.9 b	6.6±3.6 cd
Pd		NH	40.0±3.6 d	8.3±3.7 d	0.0±0.0 d
		HW	56.6±4.4 c	15.0±3.8 d	0.0±0.0 d
Pe	SV	NH	81.6±5.3 ab	63.3±5.4 a	30.0±8.5 abc
		HW	95.0±3.5 a	68.3±6.3 a	50.0±10.2 a
Pc		NH	70.0±3.3 b	26.6±2.7 c	8.3±4.4 cd
		HW	76.6±6.6 b	38.3±2.5 b	6.6±3.6 cd
Pd		NH	51.6±5.8 c	25.0±2.7 c	1.6±1.6 d

	HW	66.6±4.9 c	33.3±3.5 b	1.6±1.6 d

GT = Grafting technique; MH = Method of healing; *SE* = Standard error; TC = Terminal cleft; SV = Side veneer; Pe = *Pinus engelmannii* Carrière; Pc = *Pinus cooperi* C. E. Blanco; Pd = *Pinus durangensis* Martínez; NH = Natural healing; HW = Healing with wax. Different letters indicate significant statistical differences between interactions according to Dunnett's median comparison test.

The effect of healing waxes on graft survival varies according to the species studied (Ungureanu *et al*., 2013). For example, Blada and Panea (2011; 2012) studied the effect of the application of wax based on bioregulators in the grafting zone in the grafted area in *Picea pungens* var. *glauca* and *Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr.; their results indicated a survival of 87 and 96 %, respectively, at five months of age of the grafts, which were maintained under greenhouse conditions at an average temperature of 25 °C.

In the three assessed species, the highest survival was obtained in side-veneer grafts, which may be indicative of a greater ability of the grafter to develop this technique. However, it is possible that the conifer species studied have more affinity to be propagated by other propagation methods.

Conclusions

The species with the best response to grafting is *P. engelmannii*; side-veneer technique and the use of healing wax increased graft survival. In the case of *P. cooperi* and *P.*

durangensis, it is recommended to evaluate other factors that could favor the survival of grafts, or otherwise, explore other methods of vegetative propagation.

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Conflict of interest

The authors declare that they have no conflict of interest.

Contributions for author

Alberto Pérez-Luna: execution and supervision of the research, data capture, statistical analysis and interpretation of results and writing of the manuscript; Jesús Alejandro Soto-Cervantes and Rosa Elvira Madrid-Aispuro: statistical analysis, interpretation of results and review of the manuscript; José Ángel Sigala-Rodríguez: methodological design and review of the manuscript; Santiago Solís-González: selection of superior trees, collection of scion and revision of the manuscript; José Ángel Prieto-Ruíz: methodological design, execution and supervision of the research, verification of results and review of the manuscript.

References

Almqvist, C. 2013. Survival and strobili production in topgrafted scions from young *Pinus sylvestris* seedlings. Scandinavian Journal of Forest Research 28(6):533-539. Doi: 10.1080/02827581.2013.803598.

Aparicio-Rentería, A., H. Viveros-Viveros y V. Rebolledo-Camacho. 2013. Huertos semilleros clonales: una alternativa para los programas de reforestación en Veracruz. Revista Mexicana de Ciencias Forestales 4(20):90-97. Doi: 10.29298/rmcf.v4i20.373.

Barrera-Ramírez, R., J. J. Vargas-Hernández, R. López-Aguillón, H. J. Muñoz-Flores, E. J. Treviño-Garza and O. A. Aguirre-Calderón. 2021. Impact of external and internal factors on successful grafting of *Pinus pseudostrobus* var. *oaxacana* (Mirov) Harrison. Revista Chapingo Serie Ciencias Forestales y del Ambiente 27(2):243-256. Doi: 10.5154/r.rchscfa.2020.05.037.

Blada, I. and T. Panea. 2011. Improvement of grafting procedures for the ornamental species: I. *Picea pungens* Engelm. var. *glauca* Regel. Annals of Forest Research 54(2):185-196. https://www.afrjournal.org/index.php/afr/article/viewFile/89/135. (21 de octubre de 2022).

Blada, I. and T. Panea. 2012. Improvement of grafting procedures for the ornamental species: II. *Abies concolor* [(Gord. & Glend.) Lindl]. Annals of Forest Research 55(1):25-31. https://www.afrjournal.org/index.php/afr/article/viewFile/72/107. (21 de octubre de 2022).

Castro-Garibay, S. L., Á. Villegas-Monter, J. López-Upton, M. Sandoval-Villa and L. Arévalo-Galarza. 2022. Effective protocol to increase the percentage of grafting success of *Pinus greggii* Engelm. var. *australis* Donahue et López. Revista Chapingo Serie Ciencias Forestales 28(2):225-240. Doi: 10.5154/r.rchscfa.2021.03.014.

Chávez I., C. 2016. Injertado de corona en tres especies del género *Pinus* en la región de el Salto, Pueblo Nuevo, Durango. Tesis de Maestría. Facultad de Ciencias Forestales. Universidad Juárez del Estado de Durango. Durango, Dgo., México. 68 p.

Cuevas C., J. C. 2014. Propagación Vegetativa de *Pinus leiophylla* Schiede ex Schltdl. et Cham. Tesis de Maestría. Postgrado en Ciencias Forestales. Colegio de Postgraduados, Campus Montecillo. Texcoco, Edo. Méx., México. 72 p.

García A., A. y M. S. González E. 2003. Pináceas de Durango. Instituto de Ecología, A.C. Xalapa, Ver., México. 179 p.

González-Jiménez, B., M. Jiménez-Casas, J. López-Upton, M. Á. López-López and R. Rodríguez-Laguna. 2022. Combination of grafting techniques to clone superior genotypes of *Pinus patula* Schiede ex Schltdl. *et* Cham. Agrociencia 56(5):1-12. Doi: 10.47163/agrociencia.v56i5.2582.

Hibbert-Frey, H., J. Frampton, F. A. Blazich, D. Hundley and E. Hinesley. 2011. Grafting fraser fir (*Abies fraseri*): effect of scion origin (crown position and branch order). HortScience 46(1):91-94. Doi: 10.21273/HORTSCI.46.1.91.

Martínez S., M. y J. Á. Prieto R. 2011. Determinación de áreas potenciales para el establecimiento de plantaciones comerciales forestales en la región norte de México. Folleto Técnico Núm. 47. INIFAP-Campo Experimental Valle del Guadiana. Durango, Dgo., México. 35 p.

McKeand, S. E., J. B. Jett, J. R. Sprague and M. N. Todhunter. 1987. Summer wax grafting of loblolly pine. Southern Journal of Applied Forestry 11(2):96-99. Doi: 10.1093/sjaf/11.2.96.

Muñoz F., H. J., J. Á. Prieto R., A. Flores G., T. Pineda O. y E. Morales G. 2013. Técnicas de injertado "enchapado lateral" y "fisura terminal" en *Pinus pseudostrobus* Lindl. Folleto Técnico Núm. 68. INIFAP-Campo Experimental Valle del Guadiana. Durango, Dgo., México. 48 p.

Pérez-Luna, A., J. Á. Prieto-Ruíz, J. López-Upton, A. Carrillo-Parra, … and J. C. Hernández-Díaz. 2019. Some factors involved in the success of side veneer grafting of *Pinus engelmannii* Carr. Forests 10(2):112-229. Doi: 10.3390/f10020112.

Pérez-Luna, A., C. Whenkel, J. Á. Prieto-Ruíz, J. López-Upton, … and J. C. Hernández-Díaz. 2020a. Grafting in conifers: A review. Pakistan Journal of Botany 52(4):1369-1378. Doi: 10.30848/PJB2020-4(10).

Pérez-Luna, A., C. Wehenkel, J. Á. Prieto-Ruíz, J. López-Upton and J. C. Hernández-Díaz. 2020b. Survival of side grafts with scions from pure species *Pinus engelmannii* Carr. and the *P*. *engelmannii* × *P*. *arizonica* Engelm. var. *arizonica* hybrid. PeerJ 8: e8468. Doi: 10.7717/peerj.8468.

Pérez-Luna, A., J. C. Hernández-Díaz, C. Wehenkel, S. L. Simental-Rodríguez, J. Hernández-Velasco and J. Á. Prieto-Ruíz. 2021. Graft survival of *Pinus engelmannii* Carr. in relation to two grafting techniques with dormant and sprouting buds. PeerJ 9:e12182. Doi: 10.7717/peerj.12182.

R Core Team. 2020. The R Project for Statistical Computing (versión 3.5.3). Vienna, Austria. The R foundation for statistical computing. https://www.R-project.org/. (21 de octubre de 2022).

Rosier, C. L., J. Frampton, B. Goldfarb, F. C. Wise and F. A. Blazich. 2005. Stumping height, crown position, and age of parent tree influence rooting of stem cuttings of Fraser fir. HortScience 40(3):771-777. Doi: 10.21273/HORTSCI.40.3.771.

Solorio-Barragán, E. R., P. Delgado-Valerio, A. Molina-Sánchez, V. Rebolledo-Camacho and M. Á. Tafolla-Martínez. 2021. Interspecific grafting as an alternative for asexual propagation of *Pinus rzedowskii* Madrigal & Caball. Del. in danger extinction. Revista Chapingo Serie Ciencias Forestales y del Ambiente 27(2):277-288. Doi: 10.5154/r.rchscfa.2020.06.046.

Stewart, J. F., R. Will, B. S. Crane and D. Nelson. 2017. Occurrence of shortleaf x loblolly pine hybrids in shortleaf pine orchards: implications for ecosystem restoration. Forest Science 63(2):225-231. Doi: 10.5849/forsci.15-167.

Ungureanu, S., T. Panea and V. Ungureanu. 2013. Mijloace noi pentru creştere randamentului şi îmbunătăţirea calităţii materialului săditor de măr prin altoirea la masă. Horticultură, Viticultură şi vinificaţie, Silvicultură şi grădini publice, Protecţia plantelor 36(1):30-33. https://ibn.idsi.md/sites/default/files/imag_file/30-33_10.pdf. (21 de octubre de 2022).

White, G., W. J. Lowe and J. Wright. 1983. Paraffin grafting techniques for loblolly pine. Southern Journal of Applied Forestry 7(3):116-118. Doi: 10.1093/sjaf/7.3.116.

Yuan, H., S. Niu, X. Zhou, Q. Du, Y. Li and W. Li. 2016. Evaluation of seed production in a first-generation seed orchard of Chinese pine (*Pinus tabuliformis*). Journal of Forestry Research 27(5):1003-1008. Doi: 10.1007/s11676-016-0238-x.

Zobel, B. and J. Talbert. 1984. Applied forest tree improvement. John Wiley. New York, NY, United States. 505 p.