Graft survival of *Pinus engelmannii* Carr. in relation to two grafting techniques with dormant and sprouting buds

Abstract

Developing methods for successfully grafting forest species will be helpful for establishing asexual seed orchards and increasing the success of forest genetic improvement programs in Mexico. ~~In this study, we investigated t~~The effects of ~~the~~ grafting technique (side veneer and top cleft) and ~~the~~ phenological stage of the buds on the scions (end of latency and beginning of sprouting), in combination with seven grafting variables, on the sprouting and survival of 120 intraspecific grafts of *Pinus engelmannii* Carr were investigated in this study. The scions used for grafting were taken from a 5.5-year-old forest plantation. The first grafting was performed ~~carried~~ on ~~18~~ January 18 (buds at the end of dormancy) and the second on ~~21~~ February 21 (buds at the beginning of sprouting). The data were examined by analysis of variance and a test of means and were fitted to two survival models (the Weibull accelerated failure time ~~model~~ and the Cox proportional hazards models) and the respective hazard ratios were calculated. Survival was highest in grafts made by top cleft grafting with buds at the end of the latent period, with 80% sprouting and an estimated average survival time of between 164 and 457 days after the six-month evaluation period.~~Survival was highest in the grafts made by top cleft grafting with buds at the end of the latent period, with 80% sprouting and estimated average survival time of these grafts of between 164 and 457 days after concluding the evaluation period of six months.~~ Four variables (grafting technique, phenological stage of the scion buds, scion diameter, and rootstock height) significantly affected the risk of graft death in both survival models. Top cleft grafts with buds at the end of the latency stage, combined with scion diameters less than 11.4 mm and rootstock heights greater than 58.5 cm, had a lower risk of death.~~Four variables (grafting technique, phenological stage of the buds, scion diameter and rootstock height) significantly affected the risk of death of the grafts, in both survival models. The risk of death decreased in top cleft grafts with buds at the end of the latency stage, combined with scions of diameter less than 11.4 mm and rootstocks of height greater than 58.5 cm.~~

Key ~~W~~words Asexual propagation, Weibull accelerated failure time model, Cox proportional hazard model, Hazard ratio, Risk of death

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For example:
- Apache pine
- Cleft grafting
- side veneer grafting
- Grafting survival
- Statistical survival analysis models
- etc.

Introduction

Each year, ~~Mexico loses~~ between 75,000 and 500,000 ha of temperate and tropical forests are lost in Mexico *(Rosete-Vergés et al., 2014; Castro-Salazar & Luyando-Cuevas, 2019)*, mainly due to overexploitation, forest fires, cattle ranching, clandestine logging, adverse weather conditions and ~~change~~ a shift in land use from forest to grassland *(FAO-CONAFOR, 2009)*. Between 1970 and 2014, ~~D~~deforestation caused the loss of more than 23,000,000 ha of tropical forest and more than 13,000,000 ha of temperate forest ~~in the period 1970-2014~~ *(SEMARNAT,* *2018)*. These figures place Mexico among the first countries worldwide in terms of forest area loss ~~of forest area~~.

Mexico also has an annual deficit of 11,619,300 m^3 of round and manufactured wood, as well as a deficit of 6,535,500 m^3 of cellulosic products *(SEMARNAT, 2017)*. The constant demand for timber products from North America leads to consumption of three times more forest products than are produced in Mexico *(SEMARNAT, 2017)*. By contrast, the United States of America annually consumes 20% more wood than is produced nationally *(Fiedler et al., 2001)*.

Considering the deterioration of forests, the increased demand for wood products and the projected further 33% increase in demand for timber products in the same region ~~from~~ by 2030 *(Perlis, 2009)*, seeking ways ~~of~~ to increase~~ing~~ the supply of wood from forest resources in Mexico is imperative. In this respect, commercial forest plantation programs are particularly important *(Martínez & Prieto, 2011)*. However, there are several deficiencies in the production chain ~~of~~ for this option, ~~such as~~including a ~~the~~ lack of high quality genetic ~~quality~~ germplasm, among other factors *(Vargas & López, 2017)*.

In the state of Durango, Mexico, between 0.9 and 1.4 million hectares of forest cover were lost in the period 1986-2012 due to various factors, including forest fires, land use change, overgrazing and over-exploitation of forests *(Novo-Fernández et al., 2018)*. Fortunately, the state has available 1,150,000 ha of land with a high potential for use in establishing commercial forest plantations *(Martínez & Prieto, 2011)*. *Pinus engelmannii* Carr. is a promising candidate for this purpose, as it is one of the most economically and environmentally important species, mainly due to the high quality of its wood *(Prieto et al., 2004; González-Orozco et al., 2018)*.

Establishment of forest plantations in previously non-forested land can contribute to the conservation of natural forest resources *(Ramírez & Simonetti, 2011)* and to generating environmental services, such as carbon sequestration *(Miehle et al., 2006; Soto-Cervantes et al., 2020)*, water harvesting, soil retention *(Sayer & Elliott, 2005)* and landscape improvement *(Sabogal, Besacier & McGuire, 2015)*, among others *(Martínez & Prieto, 2011)*.

The success of commercial forest plantations depends on the use of high quality genetic material, which can be obtained from asexual seed orchards *(Yuan et al., 2016; Stewart et al., 2016; Pérez-Luna et al., 2020a)*. To establish this type of orchard, superior genotypes must first be multiplied~~it is first necessary to multiply superior genotypes~~, either by grafting, air layering, ~~rooting by~~ cutting~~s~~ or by in vitro propagation *(Iglesias, Prieto & Alarcón, 1996; Bonga, 2016)*.

Of these propagation methods, grafting is the most commonly used for cloning superior genotypes of forest species *(Stewart et al., 2016)*. Grafts can be made with scions taken from identified superior trees in natural environments and then placed on rootstocks (branches or rhizomes) from other trees, generally produced in a nursery, thus giving rise to new plants *(Jayawickrama, Jett & McKeand, 1991; Muñoz et al., 2013)*.

Graft success is influenced by factors such as~~The factors that influence the success of grafts include the~~ grafting technique, ~~the~~ phenological stage of ~~the~~ buds on ~~the~~ grafted scions ~~grafted~~ *(Jayawickrama, Jett & McKeand, 1991)*,~~the~~ genetic, anatomical and taxonomic compatibility between scions and rootstocks *(Pina & Errea, 2005)*, efficient nutrition *(Mutabaruka, Cook &*

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Buckley, 2015), grafter training and skill, ~~of the grafter~~ and the graft development environment *(Muñoz et al., 2013)*.

Survival equations, such as the Weibull's accelerated failure time model and its hazard function, enable estimation of the probability of success and prediction of the survival time following the last evaluation of the study participants ~~after the last evaluation carried out on the study individuals~~ *(Zhang, 2016)*. However, this model has mainly been used in the field of medicine *(Chaou et al., 2017)*, with limited application in studying the success of tree species grafting.~~and its application in studying the success of tree species grafting is limited.~~ The only relevant study that we are aware~~know~~ of is that ~~carried out by~~of *Pérez-Luna et al. (2020b)*.

Another widely used non-parametric survival model, especially in medical science, is the Cox proportional hazards model and the associated hazard ratio, which measures the risk of death of a group of individuals subjected to two or more treatments *(Meira-Machado et al., 2013; Gandrud 2015)*. The potential use of this model to study the effect of several factors on the survival of grafts has also recently been demonstrated by *Pérez-Luna et al. (2019)*, who suggest that these techniques are widely applicable in forestry research.

The objectives of the present study were to evaluate the effect of the grafting technique and the phenological stage of the scion buds on the percentage graft survival of *P.~~inus~~ engelmannii*. In addition, the potential effects of another seven variables on graft survival were evaluated. For this purpose, a Weibull's accelerated failure time model and the associated hazard ratio were tested, and the Cox proportional hazards model was fitted to the data. Given the scarce use of these models in the field of forestry, another important objective of this work was the validation of the usefulness of these models in explaining the effect of diverse factors on the survival of *P. engelmannii* grafts.

Materials & Methods

Rootstock production

The rootstocks were produced in the "General Francisco Villa" forest nursery in Durango, Mexico (23° 58' 20" North and 104° 35' 56" West, at 1,875 m elevation). During the first year, the rootstocks were grown in polystyrene trays (each with 77 cavities of capacity 170 mL); the substrate was a mixture of equal parts of peat moss and composted pine bark. In the second year, each rootstock was transplanted into a 3.5 L black polyethylene bag, which contained equal parts of forest soil and pine bark. At age four years (July 2018), and six months before grafting, the rootstocks were transplanted in 5 L containers (bags), to favour reactivation of the root system. The substrate in these containers was forest soil, and 50 g of Multicote® 18-6-12 (N-P-K), a ~~slow release~~slow-release fertilizer (8 months), was added to each container. The rootstocks were watered every three days, with 2 L of water per plant.

Collection of scions

The scions were taken from trees in a free access plantation of *P.~~inus~~ engelmannii*, in the Ejido Aquiles Serdán, Durango, Mexico (location 23° 53' 39" North and 104° 33' 44" West and 1,898 m elevation). When the scions were obtained, ~~T~~the plantation was five years old ~~when the scions were obtained and showed~~with good adaptation and growth. The donor trees chosen had an

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Pérez-Luna et al. (2019) recently demonstrated the potential use of this model to study the effect of several factors on graft survival, implying that these techniques are widely applicable in forestry research.

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Commented [A5]: According to the manufacturer's website, it is **Multicote™ 6: 18-6-12 (N-P-K) + micronutrients with a 6 months longevity.**
Please double-check it and add and/or correct the product information in the main text.

https://www.haifa-group.com/multicote%E2%84%A2-products-nurseries

In addition, for more information about this product, please refer the manufacturer's name and website.

For example, check the below link:

https://www.haifa-group.com/multicote-6

Formatted: Font: Not Italic, Complex Script Font: Italic average height of 3 m and a diameter of 7 cm at the base of the stem.~~The selected donor trees were of average height 3 m and diameter of 7 cm at the base of the stem.~~ The scions were collected the day before grafting and placed in 72 L plastic boxes; each layer of scions was covered with sawdust wetted with a solution of 3 g L^{-1} of Captán® fungicide, to prevent fungal damage.

Grafting

The grafting was carried out in the nursery of the Institute of Forestry and Wood Industry of the Universidad Juárez del Estado de Durango (ISIMA-UJED), in a greenhouse of dimensions 6 ×~~x~~ 8 ×~~x~~ 3 m (width ×~~x~~ length ×~~x~~ height), covered with white plastic (caliber 720). Two shading meshes (providing 50% and 70% cover) were placed in the upper part of the greenhouse, 30 cm above the plastic cover, to prevent excessive increases in temperature and to favour adequate relative humidity. In addition, two air conditioning systems were installed at the ends of the greenhouse, enabling an average temperature of 22 °C (maximum, 26 °C and minimum, 7 °C) to be maintained; the relative humidity fluctuated between 72 and 82%.

Treatments evaluated

The grafting methods evaluated were the side veneer technique described by *Muñoz et al. (2013)* and *Pérez-Luna et al. (2019)* and the top cleft technique described by *Muñoz et al. (2013)*. For each technique, two phenological stages of the buds were also tested. Half of the grafts were made with scions on which the buds were at the end of their dormancy (grafted on January 18, 2019) and the other half were made with scions on which the buds were at the beginning of the sprouting stage (grafted on ~~21~~ February 21, 2019). In total, four treatments were evaluated, with 120 grafts (60 of side veneer and 60 of top cleft) and in each type of graft, 30 grafts of each phenological condition of the buds were made.

To carry out the side veneer graft~~s~~, a longitudinal cut of 6 cm was made on one side of the scion and a wedge of approximately one centimetre was formed with a cut at the lower end of the other side (Fig. 1A). A lateral cut was made in the rootstock, of the same length as the cut on the scion, and a one-centimetre slit was left at the end of the cut on the rootstock, into which the scion wedge was inserted and tied (Fig. 1B).

For top cleft graft~~s~~, two longitudinal 6 cm cuts were made at the bottom and opposite sides of the scion, ending in a wedge shape; the central leader was eliminated from the rootstock, and a central cut (fissure) was made to an approximate depth of 6 cm, into which the scion wedge was inserted (Fig. 1C), and both components of the graft were tied together (Fig. 1D).

The grafts were tied (both techniques) with transparent rubber tapes and sealed with vinyl paint mixed with 3 g L^{-1} of Captán® fungicide, to prevent pathogens entering the graft union. Finally, a 5 L transparent plastic bag was placed around the grafted area, into which 1 L of water was poured to generate a high humidity microenvironment (Fig. 1E); in addition, each graft was covered with a kraft paper bag, which provided protection from solar radiation (Fig. 1F).

The grafts were watered every three days with plain water. In addition, from the third month of evaluation, Promyl® fungicide was added to the irrigation water (2 g L^{-1}) every eight days, to prevent fungal damage. To compensate for possible nutrient deficiency due to the loss of

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To prevent excessive temperature increases and to promote adequate relative humidity, two shading meshes (providing 50% and 70% cover) were placed in the upper part of the greenhouse, 30 cm above the plastic cover.

mycorrhiza~~,~~ caused by ~~the~~ fungicide application ~~of fungicide~~, fertigation was applied during the six~~-~~month~~s~~ ~~of~~ evaluation period~~,~~ by adding a Triple 19 water-soluble fertilizer (N-P-K) (3 g L^{-1}) to the water, which was applied in a 7 L manual watering can.

> **Commented [A7]:** For more information about this product, please refer its trade name, the manufacturer's name and website.

Experimental design and variables evaluated

The treatments were applied in a 2 ×~~x~~ 2 factorial experimental design (2 grafting techniques ×~~x~~ 2 phenological stages of the buds). Each experimental unit consisted of 10 grafts, with three repetitions per treatment. Before grafting, seven internal grafting variables of the scion and rootstock were measured (Table 1).

Sprouting and survival of the grafts were evaluated monthly for six months, and the data were examined by the Kolmogorov-Smirnov test to evaluate the normality of the variables evaluated. Analysis of variance (ANOVA) was used to detect potential significant differences between treatments. ~~-~~When significant differences were indicated and the variables were normally distributed, a post hoc Tukey's means test was carried out, with an initial confidence interval of 95% ($\alpha = 0.05$). Bonferroni correction was applied to reduce the probability of making a type I error *(Garamszegi, 2006; Napierala, 2012)*; the corrected significance value was $\alpha = 0.0125$. In addition, Student's t-tests were used to determine any significant differences in graft survival due to different levels of the independent factors, ~~i.e.~~i.e., side veneer grafts vs top cleft grafts and bud grafts at the end of latency vs bud grafts at the beginning of sprouting. In order to reduce the effect of extreme observations, before performing the analysis of variance and Student's t tests *(Burbidge, Magee & Robb, 1988)* the survival value of each treatment was transformed, by calculating the square root of the sine function of the survival quotients.

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> **Commented [A9]:** Suggested:
> Scions with buds at the end of latency vs scions with buds at the beginning of sprouting

Finally, the Weibull accelerated failure time model was fitted to the data to predict the estimated graft survival time after the evaluation period, and the associated hazard ratio was also calculated. Other hazard ratios were estimated using the Cox proportional hazards model. All statistical analyses were implemented in the "Survival" package in the free R software *(R Development Core Team, 2018)*.

Fitting the Weibull accelerated failure time model and its hazard ratio

To fit the accelerated failure time model to the graft survival data, the following independent variables were used: grafting technique, phenological stage of the scion buds and the seven grafting variables (Table 1). To detect the significant variables affecting the estimated survival time of the grafts, stepwise regression was applied using the "StepReg" package in R *(R Development Core Team, 2018)*. The Weibull accelerated failure time model is defined as follows:

$$Ln(T) = \alpha + \delta x_i + \sigma\varepsilon \qquad (1)$$

where *Ln (T)* is the natural logarithm of the mean survival time *(T)* after the study, ~~i.e.~~i.e., at time *(T)* at least one death may occur among the grafts that were alive at the end of the evaluation period; α is a scale parameter of the model, δ is the coefficient of the explanatory variable, σ is the shape parameter of the model and ε is the error of the distribution function *(George, Seals & Aban, 2014; Zhang, 2016)*. The Weibull hazard ratio is described as follows:

$$HR_W = (e^{-\beta})^{\lambda-1} \quad (2)$$

where HR_W is the Weibull hazard ratio, which represents the increase or decrease in the risk of death of grafts as a function of the independent variables; β is a coefficient that indicates the effect of a given independent variable (from the same *xi* variables used in Eq. 1). The value of the dependent variable is not used in equation (2), because the Weibull hazard ratio is considered to be "constant for each variable", i.e., there is only one hazard ratio value for each explanatory variable in the model *(Igl, 2018)*. The *β* coefficient is interpreted as follows: if β is positive the risk of death decreases as the value of the independent variable decreases, and if β is negative, the risk of death decreases as the value of the independent variable increases *(Carroll, 2003)*. Finally, λ is a shape parameter of the model. If $\lambda > 1$ the hazard ratio increases, and if $\lambda < 1$ the hazard ratio decreases *(Zhang, 2016)*. The β and λ parameters were calculated with flexible parametric regression, which is useful for modelling survival from a time of origin to the instant at which an event occurs (life or death) *(Igl 2018; Pérez-Luna et al., 2020b)*.

The Weibull hazard ratio can take values ranging between 0 and ∞ *(Ruíz, 2012)*. A hazard ratio of 0 indicates that the risk of death decreases by 100% [(0-1) * 100)]. A hazard ratio of > 0 or < 1, for example $HR_W = 0.5$, indicates that the risk of death due to the effect of a variable is reduced by 50% [(0.5-1) * 100)] when the level of that particular variable changes. On the other hand, if the hazard ratio is 1.0, the risk of death does not vary due to the effect of changes in some particular variable [(1-1) * 100)]; and if the hazard ratio takes a value of 2, the risk of death doubles, i.e. the risk of death increases by 100% due to the effect of changes in the explanatory variable [(2-1) * 100)] *(Hilsenbeck et al., 1998; Spruance et al., 2004)*. Interpretation of the hazard ratio is similar for values greater than 2 *(Ruíz, 2012)*. It is important to take into account that the Weibull hazard ratio is calculated individually for each dependent variable in the model *(Zhang, 2016)*.

For fitting these models, dummy variables were used to code the dependent variable (survival) and the independent variables (grafting technique and phenological stage). Therefore, a live graft was coded as 0 and a dead graft as 1 (censor variables). The coding of the grafting technique was 1 for side veneer grafts and 2 for top cleft grafts; the phenological stage was coded as 1 for cuttings with buds at the end of dormancy and 2 cuttings with buds at the beginning of sprouting.

For more details on the Weibull accelerated failure time model and its hazard ratio, see *Pérez-Luna et al. (2020b)*.

Fitting the Cox proportional hazards model

The variables shown in Table 1 were used to fit this model, and the most significant variables were selected by stepwise regression. The Cox proportional hazards model used for calculating hazard ratios is calculated as follows:

$$HR_C = e^{(\varphi_1 x_{i1} + \cdots + \varphi_k x_{ik})} \quad (3)$$

where HR_C is the Cox hazard ratio expressed as a function of the independent variables (*xi*) with which the model is fitted in order to predict the increase or decrease in the risk of death of the individuals under study (grafts). φ represents the fit parameters of the model, up to the k-th independent variable. If φ is positive, the hazard ratio increases when the value of x increases (decreasing the probability of survival); if the value of φ is negative, the hazard ratio decreases when x increases (increasing the probability of survival). The range of values and the interpretation of the Cox hazard ratio are the same as for the Weibull hazard ratio. The Cox hazard ratio value is calculated globally, i.e. by including all the independent variables at one time in the HR_C equation *(Ata & Sözer, 2007)*.

Several authors recommend the use of hazard ratios derived from the Weibull model, as long as the shape parameter of the model (λ), which must be calculated before using the hazard ratio *(Lee and Wang, 2003; Lawless, 2011)*, is known; however, its counterpart, the hazard ratio of the Cox's proportional hazards model, does not depend on the evaluation time or predicted survival and therefore has the advantage of being less restrictive than the HR_W *(Cox, 1972)*.

To calculate the hazard ratio for graft survival using the Cox proportional hazards model, dummy variables, including censor variables (dead grafts), were also used, so that the coding for the dependent variables was the same as in the model of Weibull. Further details on the use of this model to assess graft survival are given by *Pérez-Luna et al. (2019)*.

Results

The results of the survival analyses, although of a different nature, were congruent with the estimated hazard ratios, as can be seen in the results described below.

Survival

Six months after grafting, the average survival rate of grafts made ~~with~~ using the top cleft and ~~the~~ side veneer techniques were 56.7% and 18.3%, respectively, with significant differences between techniques. There were also differences ~~between~~ in the phenological stages of the buds, which were analyzed as individual factors (Table 2 and Fig. 2), yielding 50% survival in ~~the~~ grafts with buds at the end of latency and 25% survival in ~~the~~ grafts with buds at the beginning of sprouting. The survival related to phenological stage was 50% in grafts made with buds at the end of dormancy and 25% in grafts with buds at the beginning of sprouting. The analysis of variance ~~results~~ indicated significant statistical differences (before the Bonferroni correction) due to the effect of these two treatments (Table 2); however, after the Bonferroni correction only the grafting technique was statistically significant. Furthermore, the Tukey test revealed that the best interaction was the combination of top cleft grafts with scions having buds at the end of the latency stage, with 80% survival, while the treatment yielding lowest survival (16.7%) was the side veneer grafts with scions having buds at the beginning of sprouting (Fig. 3).

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Fitting the Weibull accelerated failure time model and its hazard ratio

The stepwise regression indicated that the Weibull accelerated failure time model can describe survival with only four independent variables, for which the lowest value of the Akaike information criterion (AIC) was achieved (Table 3). The model fit was highly significant, even after Bonferroni correction (p <0.0001), for predicting the mean graft survival time after the six-month evaluation period.

In addition, all parameters estimated for the fitting this four-variable model were significant for the original critical level proposed at the beginning (p <0.05), and only the phenological stage of the buds was significant after the Bonferroni correction (p <0.0125) (Table 4). The estimated parameters for fitting the Weibull risk function are shown in Table 5.

Application of the accelerated failure time model (Eq. 1) to the data showed that the estimated time of survival of the grafts was greater for those produced by the top cleft technique ($x = 2$) and with buds at the end of latency ($x = 1$). In addition, the estimated survival time was greater when the diameter of the scion was less than 11.4 mm ($x \leq 1$) and the height of the rootstock was greater than 58.5 cm ($x \geq 1$). The longest estimated average survival time, after the observation period (six months), was 457 days, while the shortest estimated average survival time was 164 days.

The estimates depend on the combinations of the values of each independent variable, and these results represent the estimated time during which at least one death could occur among the grafts that were alive at the end of the evaluation period *(Zhang, 2016; Pérez-Luna et al., 2020b)*.

A negative sign of the β_1 coefficient indicates that when the variable "grafting technique" increases by one unit, from $x = 1$ (side veneer graft) to $x = 2$ (top cleft graft), the risk of death of at least one graft will be reduced by 76% [(HR_W-1) * 100 = (0.24-1) * 100 = -76%].

Interpretation of the negative value of the β estimator of the variable "rootstock height" is the same as the case described above; therefore, for rootstocks of height greater than 58.5 cm, the risk of death of the grafts is reduced by 4% [(HR_W -1) * 100 = (0.96-1) * 100 = -4%]. On the other hand, the positive signs of β for the variables "phenological stage of buds" and "diameter of scion" indicate that by increasing the respective variable by one unit, the risk of death of the grafts will increase according to the value obtained for each hazard ratio; thus, in the case of the "phenological stage of buds" for $x = 2$ (grafts with buds at the beginning of sprouting), the risk of death increased by 64% [(HR_W-1) * 100 = (1.64-1) * 100 = 64%] when the phenological stage is $x = 1$ (grafts with buds of scions at the end of dormancy). Finally, for scions of diameter greater than 13.38 mm, the risk of death of the grafts increased by 25% [(HR_W-1) * 100 = (1.25-1) * 100 = 25%].

Fitting the Cox proportional hazards model

Using stepwise regression, the variables that best explained the hazard ratio in graft survival were also selected by fitting the Cox proportional hazards model and it was confirmed that the grafting technique, the phenological stage of the buds, the rootstock height and the scion diameter were the outstanding variables for the best fit (Table 6).

The variables that best explained the hazard ratio of the Cox proportional hazards model were the same variables selected for fitting the Weibull accelerated failure time model, and although Tables 3 and 6 are similar, note that the value of the Akaike's information criterion was lower in Table 6 (AIC = 609.6) than that shown in Table 3 (AIC = 904.8). It thus follows that the prediction of survival probability is better when estimated with the Cox proportional hazards model than with the Weibull model. The estimators calculated for fitting the Cox model are shown in Table 7.

The negative values of the estimators of the coefficients of the explanatory variables in Table 7 appeared in the same variables that had already been detected when calculating the Weibull risk function ("grafting technique" and "height of rootstock") (Table 5). Interpretation of the algebraic signs of the estimators is similar in both tables and is based on the interpretation of Table 5, which refers to the risk of death of the grafts. Therefore, the negative values of the estimators shown in Table 7 also indicate that the risk of death of the grafts decreases when the value of these variables increases, i.e. when $xi > 1$ (top cleft grafting technique and when the height of the rootstock is greater than the mean value observed, i.e. 58.5 cm, the risk of death decreases) and therefore, the probability of survival increases. On the other hand, the positive values of the variables "phenological stage of buds" and diameter of scion" indicate that the risk of death increases when $xi > 1$ (grafts with buds at the beginning of sprouting and scions with diameters greater than the observed mean value, i.e. 11.4 mm).

The hazard ratio values (Table 8) were calculated using equation (3). It was estimated that the lowest value of the hazard ratio of graft death (0.18) was obtained for the top cleft grafts with buds at the end of the dormancy period, using rootstocks taller than 58.5 cm and scions of diameter less than 11.4 mm; this value implies that the risk of death decreases by 82% [$(HR_C-1) * 100 = (0.18-1) * 100 = -82\%$] when grafts are produced with this combination of variables, relative to the hazard ratio obtained for other combinations. On the other hand, the highest estimated hazard ratio corresponded to the side veneer grafts with buds at the beginning of sprouting, for rootstocks shorter than 58.5 cm and scions of diameter greater than 11.4 mm, taking a value of 2.0, which indicates that the risk of death under this combination increases by 100% [(HR_C-1) ×* 100; (2-1) ×* 100 = 100%], relative to the other combinations.

Discussion

In a recent study in Durango, Mexico, *Pérez Luna et al. (2019)* performed side veneer grafts with 5 to 7-year-old rootstock of *Pinus engelmannii*, under normal greenhouse conditions (without automatic climate control) and reported survival of 22.5% six months after grafting, with no significant differences between grafting with scions having buds at the end of dormancy or at the beginning of sprouting. Regarding the side veneer grafting technique, the results were similar to those obtained in the present study, and therefore the climate control applied in the present work does not seem to have influenced the survival in the side veneer technique. On the other hand, the better survival of the top cleft grafts obtained in the present study may be related to the more favourable environment (temperature and relative humidity) inside the greenhouse.

In side veneer grafts of *Pinus greggii* Engelm var. *australis* Donahue & López in Veracruz, survival greater than 60% after three months was reported, which was partly attributed to the fact that both the seedlings with which the rootstocks were produced and the grafted cuttings were obtained from the same geographic location *(Alba-Landa et al., 2017)*. In the present study, the scions and rootstocks were of different origin, which may contribute to explaining the low percentage of successful grafts with this technique. On the other hand, *Wendling, Stuepp & Zuffellato-Ribas (2016)* found that grafting of *Araucaria angustifolia* (Bertol.) Kuntze was more effective with the budding or chip technique (chip budding), which is very similar to the side veneer grafting, reporting almost 40% survival at 130 days after grafting.

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Commented [A12]: Please provide a more in-depth discussion with your findings on grafting success and survival based on plant physiological and anatomical determinants. Please take note of the highlighted issues and respond to the questions in the following comments.

Commented [A13]: What do you mean by "normal" greenhouse conditions? As you are aware, a greenhouse without a controlled environment is not standard or suitable for conducting research in.
Please provide a concise and clear description of the experimental situation details in the research conducted by Pérez Luna et al (2019).

Commented [A14]: Environmental factors such as temperature and relative humidity have a significant impact on grafting success and survival. Please elaborate on your claim based on a comparison of exact environmental situations in present study with the research conducted by Pérez Luna et al (2019).

Commented [A15]: As you are aware, each of your scientific claims in the discussion section must be adequately interpreted in light of previous results reported in the literature.
Please explain how the origin of the scions and rootstocks can affect the rate of graft success when all grafts are intraspecific.

Commented [A16]: What is the similarity between chip budding and side veneer grafting? Are you referring to their site preparation? Please explain this claim and refer to its references in the text.

Mencuccini et al. (2007) recommend using rootstocks younger than two years for grafting *Pinus* species. *Dorman (1976)* pointed out that the sprouting of side veneer grafts in *Pinus* species was significantly lower when rootstocks older than three years were used. In the present study, the rootstocks were older than four years. The proved to be unfavourable for survival of side veneer grafts (18.3%), although the survival of top cleft grafts (56.7%), can be considered acceptable, despite the fact that the rootstocks were of the same ages. In another study showing the importance of the rootstock age, *Zhang & Tang (2005)* reported 50% survival for grafting *Pinus ponderosa* Douglas ex C. Lawson with two-year-old rootstocks.

Other authors have also achieved good survival when grafting conifers with the top cleft technique. For example, *Almqvist (2013a; 2013b)* reported survival rates of 75.0 and 84.7% in two *Pinus sylvestris* L top cleft grafting experiments. In *Abies fraseri* (Pursh) Poir grafts, *Hibbert-Frey et al. (2011)* reported 86% survival with the same technique. Similarly, *Singh (1992)* reported good success for grafting *Pinus gerardiana* Wall using the top cleft technique, with 70% survival.

Villaseñor & Carrera (1980) reported survival of 63.0% in top cleft grafts of *Pinus patula* Schl. et Cham. in Mexico, with scions having dormant buds. Likewise, *Świerczyński et al. (2020)* achieved survival greater than 80% for side veneer grafts of *Pinus mugo* Turra, established in winter (with scions having dormant buds). Survival greater than 60.0% was reported for top cleft grafts of *Araucaria angustifolia*, in Brazil, ~~grafted~~ using scion cuttings with dormant buds *(Gaspar et al., 2017)*. On the other hand, when grafting *A. angustifolia*, *Zanette, Oliveira & Biasi (2011)* reported only 20% and 0% survival in grafts with buds at the beginning of dormancy (spring) ~~and 0% survival when grafting *A. angustifolia* with buds~~and in full sprouting (summer), respectively. These results are consistent with those obtained in the present study and, although different species of the order Pinales were used, they must share certain characteristics in terms of their phenological functioning *(Bodnar et al., 2015)*.

To analyze graft survival, *Pérez-Luna et al. (2020b)* fitted the Weibull accelerated failure time model to data on side veneer grafts of *Pinus engelmannii*, estimating an average survival time of 154 days after the end of the six-month evaluation period. The higher values of potential survival time found in the present study can be attributed to ~~the fact that the present study included~~ automated temperature and environmental humidity controls in the greenhouse where the grafts were held, but not in the previous study.

The effects of the grafting variables considered in the present study were also evaluated in the aforementioned study *(Pérez-Luna et al., 2020b)*, and it was found that the variables did not significantly affect the risk of graft death ~~of the grafts~~. The significant effects observed in the present study is in ~~These results~~ contrast with their results ~~the significant effects observed in the present study~~, which can be attributed to the fact that on this occasion the climatic conditions inside the greenhouse were controlled, reducing the variation caused by factors not evaluated and, therefore, the survival of the grafts can be more directly attributed to their response to the treatments and to the evaluated grafting variables. In a grafting experiment with *Araucaria angustifolia*, *Wendling, Stuepp & Zuffellato-Ribas (2016)* obtained survival greater than 20%

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Are you referring to sprouting?

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after 130 days of evaluation for rootstocks of between 80 and 100 cm in height; our findings indicate that the survival of *P. engelmannii* grafts is improved by using rootstocks taller than 55.8 cm.

Pérez-Luna et al. (2019) also used the Cox proportional hazards model and its hazard ratio to evaluate the effect of the phenological stage of the scion buds (end of latency and beginning of sprouting), observing that this factor did not have a significant influence on the graft mortality risk.

Commented [A20]: Please explain why there is such a substantial difference between your results and those reported by *Pérez-Luna et al* (2019).

Conclusions

The survival models used in medical studies were also useful tools for evaluating the success of *P. engelmannii* grafts. In the other words, the Weibull accelerated failure time and the Cox proportional hazards models and their respective hazard ratios were validated for use in predicting the survival rate (risk of death) as a function of some factors considered, such as the grafting technique, the phenological stage of buds and some grafting variables inherent to the scions and rootstocks. Although the Cox proportional hazards model provided a better fit to the data, use of the Weibull accelerated failure time model is also recommended, as it enabled reliable prediction of the estimated graft survival time after the evaluation period. The best grafting technique for asexual propagation of *Pinus engelmannii* proved to be the top cleft method, and the best phenological condition for the scions was buds at the end of the latency period. Grafting was more successful with scions of diameter less than 11.4 mm. In addition, in order to reduce the risk of death of top cleft and side veneer grafts of *P. engelmannii*, use of rootstocks taller than 58.5 cm ~~is~~ was recommended. ~~The findings also confirmed that survival models used in medical studies are also useful tools for evaluating the success of *P. engelmannii* grafts. The results can also serve as a guide for decision-making for grafting in other species. In the present study, the Weibull accelerated failure time model and the Cox proportional hazards model and their respective hazard ratios were validated for use in predicting the survival rate (risk of death) as a function of some factors considered, such as the grafting technique, the phenological stage of buds and some graft variables inherent to the scions and rootstocks. Although the Cox proportional hazards model provided a better fit to the data, use of the Weibull accelerated failure time model is also recommended, as it enables reliable prediction of the estimated graft survival time after the evaluation period.~~ Finally, ~~T~~the results of the present study can ~~also~~ serve as a guide for decision-making for grafting in other species.

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