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- 316 The variables that best explained the hazard ratio of the Cox's proportional hazards model were
- the same variables selected for fitting the Weibull's accelerated failure time model, and although
- 318 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). Therefore, the Cox's
- 320 proportional hazards model performed better than Weibull's model for predicting the survival
- 321 probability. The estimators calculated for fitting the Cox's model are shown in Table 7.
- The hazard ratio values (Table 8) were calculated using equation (3). It was estimated that the lowest value of the hazard ratio of grafts death (0.18) would be obtained for the top cleft grafts
- with the scion buds at the end of the dormancy period, using rootstocks taller than 58.5 cm and
- 325 scions with diameter smaller than 11.4 mm; this value implies that the risk of death would
- decrease by 82% [( $HR_C$ -1) × 100 = (0.18-1) × 100 = -82%] when grafts are produced with this
- 327 combination of variables, relative to the hazard ratio obtained for other combinations. On the
- 328 other hand, the highest estimated hazard ratio corresponded to the side veneer grafts using scions
- 329 with buds at the beginning of sprouting, for rootstocks shorter than 58.5 cm and scions with
- 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
- 332 combinations.

#### 333 Discussion

- In this study, a higher survival of top cleft grafts was observed (56.7%) than in side veneer grafts
- (18.3%); the two types of grafts were maintained under climate-controlled conditions within the
- 336 greenhouse. Temperature control during coniferous grafting is important to increase the survival
- 337 probability, as observed by *Blada & Panea (2011)*, who found that callus formation in side
- 338 veneer grafts of *Picea pungens* Engelm var. *glauca* Regel was favoured by temperatures between
- **339** 20 and 25 °C.
- 340 In a recent study in Durango, Mexico, *Pérez Luna et al. (2019)* performed side veneer grafting
- 341 with 5 to 7-year-old rootstocks of *Pinus engelmannii*, under greenhouse conditions without
- 342 automatic climate control, and reported survival of only 22.5% six months after grafting, with no
- 343 significant differences between grafting using scions with buds at the end of dormancy or at the
- 344 beginning of sprouting. These low survival results were attributed by the authors to the high
- temperatures registered in the greenhouse during the months of March to May (maximum
- recorded, 42.6 °C), and to the low relative humidity in the same months (on average, 38%).
- 347 However, in the present study, the survival obtained in the side veneer grafts, were similar to
- 348 obtained for *Pérez-Luna et al. (2019)*, and therefore the climate control applied in the present
- work does not seem to have influenced the survival in grafts this technique.
   indicating that climate control appears to be ineffective in this regard.
- achieved in the present study may be related to the more favourable (controlled) environment
- 352 (maximum temperature of 26 °C and minimum relative humidity of 72%) inside the greenhouse.
- 353 However, low temperatures also usually strong affect the survival of coniferous grafts, as
- observed by *Cuevas (2014)*, who reported 100% mortality in top cleft and side veneer grafts of
- 355 *Pinus leiophylla* Schiede ex Schltdl. *et* Cham for a minimum temperature of -5.8 °C.

- 356 In side veneer grafts of Pinus greggii Engelm var. australis Donahue & López in Veracruz,
- 357 survival was greater than 60% after three months, which was partially attributed to the fact that
- 358 both the seedlings with which the rootstocks were produced and the grafted scions were obtained
- 359 from the same geographic location (Alba-Landa et al., 2017). In the present study, the scions and
- 360 rootstocks were of different origin, which may contribute to explaining the low percentage of
- 361 successful grafts with this technique. In this respect, some studies did not observe any effect of
- the origin on the success of grafting; e.g. *Aparicio et al. (2009)* reported that the provenance of
- 363 the scions did not influence the survival of terminal fissure grafts of *Austrocedrus chilensis* (D.  $D_{12} = 0$
- 364 Don) Pic Serm & Bizzarri; however, *Villaseñor & Carrera (1980)* reported that the provenance
- of the scions had a significant effect on the success of the top cleft and side veneer grafting of
- 366 *Pinus patula* Schl. *et* Cham.
- 367 The genetic compatibility between the scion and the rootstock is also a determining factor in
- 368 grafting success (Lott et al., 2003; Hibbert-Frey et al., 2001); in this regard, it is important to
- 369 consider the results of recent studies (Wehenkel et al., 2020; Simental-Rodríguez et al., 2021),
- 370 which indicate large genetic differences and even hybridizations between close populations of
- 371 the genus *Pinus* in northern Mexico.
- 372 On the other hand, *Wendling, Stuepp & Zuffellato-Ribas (2016)* found that grafting of *Araucaria*
- 373 angustifolia (Bertol.) Kuntze was effective with the chip budding technique (which is similar to
- the side veneer grafting regarding the cut made in the rootstock), reporting almost 40% survival
- at 130 days after grafting.
- 376 Mencuccini et al. (2007) recommend using rootstocks younger than two years for grafting Pinus
- 377 species. Dorman (1976) pointed out that the sprouting of side veneer grafts in Pinus species was
- 378 significantly lower when rootstocks older than three years were used. In the present study, the
- rootstocks were older than four years and the survival of side veneer grafts was low (18.3%)
- despite the fact that the rootstocks used in both grafting techniques were of the same age;
- 381 however, the survival of top cleft grafts (56.7%) can be considered acceptable. In another study,
- 382 Zhang & Tang (2005) reported 50% survival in top cleft grafting of Pinus ponderosa Douglas ex
- 383 C. Lawson with two-year-old rootstocks. In the top cleft graft of *Pinus elliottii* Engelm., a
- survival rate of 30% was reported for grafting performed with two- to three-year-old rootstocks
   *(Mergen, 1955).*
- 386 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
- 388 two *Pinus sylvestris* L top cleft grafting experiments. In *Abies fraseri* (Pursh) Poir grafts,
- 389 *Hibbert-Frey et al. (2011)* reported 86% survival with the same technique. Similarly, *Singh*
- 390 (1992) reported good success when grafting *Pinus gerardiana* Wall, using the top cleft
- 391 technique, with 70% survival.
- 392 Villaseñor & Carrera (1980) reported survival of 63.0% in top cleft grafts of Pinus patula in
- 393 Mexico, using scions with dormant buds. Likewise, Świerczyński et al. (2020) achieved survival
- 394 greater than 80% for side veneer grafts on *Pinus mugo* Turra, established in winter (using scions
- with dormant buds). Survival greater than 60.0% was reported for top cleft grafts of *Araucaria*

- 396 angustifolia, in Brazil, using scions with dormant buds (Gaspar et al., 2017). On the other hand,
- 397 when grafting *A. angustifolia*, *Zanette*, *Oliveira* & *Biasi* (2011) reported only 20% and 0%
- 398 survival in grafts with buds at the beginning of sprouting (spring) and during full sprouting
- 399 (summer), respectively. These results are consistent with those obtained in the present study and,
- 400 although different species of the order Pinales were used, they must share certain characteristics
- 401 in terms of their phenological functioning (Bodnar et al., 2015).
- 402 To analyze graft survival, *Pérez-Luna et al. (2020b)* fitted the Weibull's accelerated failure time
- 403 model to data on side veneer grafts of *Pinus engelmannii*, estimating an average survival time of
- 404 154 days after the end of the six-month evaluation period. The higher values of potential survival
- time (up to 457 days) observed in the present study can be attributed to the automated
- temperature and environmental humidity controls in the greenhouse where the grafting was
- 407 conducted in the present study. By contrast, only two shading meshes (providing 70 and 50%
- light retention) were placed above the greenhouse in the previous study, to reduce the
- 409 temperature inside.
- 410 The negative values of the estimators of the coefficients of the explanatory variables in Table 7
- 411 appeared in the same variables that had already been detected when calculating the Weibull's
- risk function (for "grafting technique" and "height of rootstock") (Table 5). Interpretation of the
- 413 algebraic signs of the estimators is similar in both tables and is based on the interpretation of
- 414 Table 5, which refers to the risk of death of the grafts (*Cox, 1972*). Therefore, the negative values
- 415 of the estimators shown in Table 7 also indicate that the risk of death of the grafts decreases
- 416 when the value of these variables increases, i.e. when  $x_i > 1$ ; i.e. when using the top cleft grafting
- 417 technique and when the height of the rootstock is greater than the mean value observed (58.5
- 418 cm), the risk of death decreases and therefore, the probability of survival increases. On the other
- 419 hand, the positive values of the variables "phenological stage of buds" and diameter of scion"
- 420 indicate that the risk of death increases when xi > 1 (grafts with buds at the beginning of
- 421 sprouting and scions with diameters greater than the observed mean value, i.e. 11.4 mm).
- The effects of the other seven grafting variables considered in the present study (Table 1) were
- 423 also evaluated in the aforementioned study (*Pérez-Luna et al., 2020b*), and it was found that
- these variables did not significantly affect the risk of graft death. The significant effects observed
- 425 in the present study contrast with these previous findings, which can be attributed to the fact that
- 426 on this occasion the climatic conditions inside the greenhouse were controlled, thus reducing the427 variation caused by factors not evaluated. The survival of the grafts can therefore be more
- 428 directly attributed to their response to the treatments and to the grafting variables evaluated. In a
- 429 grafting experiment with Araucaria angustifolia, Wendling, Stuepp & Zuffellato-Ribas (2016)
- 430 obtained survival greater than 20% after 130 days of evaluation for rootstocks between 80 and
- 431 100 cm in height; our findings indicate that the survival of *P. engelmannii* grafts is improved by
- 432 using rootstocks taller than 55.8 cm.
- 433 Using scions with diameter larger than the rootstock diameter can cause localized graft
- 434 incompatibility. This effect was visualized by *Sweet & Thulin (1973)*, who reported this type of
- 435 incompatibility in grafts of *Pinus radiata* D. Don, as the cambium did not coincide between the

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- 436 grafted organs. On the other hand, *Pérez-Luna et al. (2020)* observed that when using short
- 437 rootstocks of *P. engelmannii* (less than 30 cm), the graft was also short (less than 15 cm height in
- 438 average), hampering establishment of a protective microenvironment and subsequent
- 439 management of the grafts.
- 440 Pérez-Luna et al. (2019) also used the Cox's proportional hazards model and the associated
- 441 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 risk
- 443 of graft mortality. The difference in the hazard ratio due to the effect of the phenological stage of
- the scion buds in the present study relative to the aforementioned research can mainly be
- attributed to the differences in management of the greenhouse conditions, as in the previous
- 446 study the climate control was not used.

#### 447 Conclusions

- 448 Two survival models used in medical studies proved useful tools for evaluating the success of *P*.
- 449 engelmannii grafts. Thus, the Weibull's accelerated failure time model and the Cox's
- 450 proportional hazards model and their respective hazard ratios were validated for use in predicting
- 451 the survival rate (risk of death) as a function of the factors considered, such as the grafting
- technique, the phenological stage of scion buds and some other grafting variables (Table 1)
- inherent to the scions and rootstocks. Although the Cox's proportional hazards model provided
- better fit to the data, the use of the Weibull's accelerated failure time model is also
- 455 recommended, as it enabled reliable prediction of the estimated graft survival time after the end
- 456 of the evaluation period. The best grafting technique for asexual propagation of *P. engelmannii*<sup>4</sup>
- 457 proved to be the top cleft method, and the best phenological condition for the scion buds was at
- the end of the latency period. Grafting was more successful with scions of diameter smaller than
- 11.4 mm. In addition, in order to reduce the risk of death of top cleft and side veneer grafts of *P*.
- 460 *engelmannii*, the use of rootstocks taller than 58.5 cm is recommended. The results of the present
- study can serve as a guide for decision-making for grafting in the studied species.

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#### 466 **References**

- Alba-Landa J, Mendizábal-Hernández LDC, Ramírez-García E, Márquez R J, Cruz-Jiménez H,
  Rodríguez-Juárez MC. 2017. Injertos de fenotipos selectos de *Pinus greggii* Engelm. de
  una prueba genética en Villa Aldama, Veracruz, México. *Foresta Veracruzana* 19:57-61.
- 409 una prueba genetica en vina Aldana, veracruz, Mexico. *Foresta veracruzana* 19.57-01 470 Almqvist C. 2013a. Survival and strobili production in top grafted scions from young *Pinus*
- 470 Aniquist C. 2013a. Survival and subbin production in top graned scions from young *1 ma.* 471 *svlvestris* seedlings. *Scandinavian Journal of Forest Research* 28:533-539 DOI:
- **472** 10.1080/02827581.2013.803598.
- 473 Almqvist C. 2013b. Interstock effects on top graft vitality and strobili production after top
- 474 grafting in *Pinus sylvestris*. *Canadian journal of forest research* 43:584-588 DOI:
- 475 10.1139/cjfr-2012-0507.

It is not appropriate to refer to the tables in the conclusion of the manuscript.