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Forestal Catalana's Stone Pine Grafting Program In Catalonia. Production Of Grafted Plant In Nursery.

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Abstract. The forestry sector in Spain faces the need to develop more attractive and profitable products. One of the most valuable in the Mediterranean forest is the pinenut which use in the local cuisine is ancestral. Moreover, its nutritional value is highly appreciated and currently the world demand is far from being covered. But its production still comes from naturals stands and a certain degree of domestication is required to increase the offer. First step was to select highly productive clones and the second to verify the grafted plants improve the entry into production. Now it is time to achieve a good level of grafting success to exploit the potential of these genotypes. More than 7,300 grafts were made for four years to identify factors that influence grafting success: scion quality, grafting period, clone origin, grafter experience, etc. The results shown that the average grafting success is up to 82.6% over four years, with slight variations depending on the grafter experience or climatic conditions. The importance to achieve suitable scions for grafting is highlighted, leading to the establishment of officially commissioned mother fields to produce FRM, scions of the registered genotypes. The work concludes that the main current challenge is not the grafting process itself, but the need to increase scion production to supply the current demand for Stone pine grafted plants.
Keywords: *Pinus pinea;* Grafting; Selected clones; Scion orchard; Agronomic management.

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Programa de Enxertia de Pinheiro-manso na Catalunha. Produção de plantas enxertadas em viveiro.

Resumo. O sector florestal em Espanha necessita de desenvolver produtos mais atraentes e lucrativos. Um dos mais valiosos na floresta mediterrânea é o pinhão, cujo uso na culinária local é ancestral. O valor nutricional desta semente é altamente apreciado e atualmente a demanda mundial é bastante superior à oferta. A grande percentagem da produção provém de florestas naturais, pelo que a domesticação desta cultura é necessária para aumentar a oferta. Numa primeira fase selecionaram-se clones altamente produtivos e seguidamente verificou-se se as plantas enxertadas melhoraram a entrada em produção. É agora necessário obter elevadas taxas de sucesso na enxertia para explorar o potencial destes genótipos seleccionados. Mais de 7.300 enxertos foram realizados ao longo de quatro anos para identificar fatores que influenciam o sucesso da enxertia: qualidade do garfo, período de enxerto, origem do clone, experiência do enxertador, entre outros. Os resultados mostraram que a média de sucesso na enxertia foi de até 82,6% ao longo de quatro anos, com pequenas variações dependendo da experiência do enxertador ou das condições climatéricas pós-enxertia. Destaca-se a importância de se obter garfos adequados, requerendo para tal o estabelecimento de campos de pés-mãe registados para produzir MFR, e garfos dos genótipos seleccionados. O principal desafio atual não reside no processo de enxertia em si, mas na necessidade de aumentar a produção de garfos para suprimir a demanda atual por plantas de pinheiro-manso enxertadas.

Palavras-chave: *Pinus pinea*; Enxerto; Clones selecionados; Pomar de enxertos; Manejo agronômico.

Programme de greffage de pins parasols en Catalogne. Production de plants greffés en pépinière.

Résumé. Le secteur forestier en Espagne fait face à la nécessité de développer des produits plus attractifs et rentables. L'un des plus précieux dans la forêt méditerranéenne est le pignon, dont l'utilisation dans la cuisine locale est ancestrale. De plus, sa valeur nutritionnelle est très appréciée et la demande mondiale est actuellement loin d'être satisfaite. Cependant, sa production provient encore de peuplements naturels et un certain degré de domestication est nécessaire pour augmenter l'offre. La première étape a été de sélectionner des clones hautement productifs et la seconde de vérifier que les plants greffés améliorent l'entrée en production. Il est maintenant temps d'atteindre un bon niveau de succès de greffage pour exploiter le potentiel de ces génotypes. Plus de 7 300 greffes ont été réalisées pendant quatre ans pour identifier les facteurs qui influencent le succès du greffage : qualité du greffon, période de greffage, origine du clone, expérience du greffeur, etc. Les résultats montrent que le taux de succès moyen des greffes atteint jusqu'à 82,6 % sur quatre ans, avec de légères variations selon l'expérience du greffeur ou les conditions climatiques. L'importance d'obtenir des greffons adaptés au greffage est soulignée, ce qui a conduit à l'établissement de champs mères officiellement commissionnés pour produire des FRM, des greffons des génotypes enregistrés. Le travail conclut que le principal défi actuel n'est pas le processus de greffage en lui-même, mais la nécessité d'augmenter la production de greffons pour répondre à la demande actuelle de plants de pin parasol greffés.

Mots-clés : *Pinus pinea* ; Greffage ; Clones sélectionnés ; Verger de greffons ; Gestion agronomique.

Introduction

In many regions of Spain, the forestry sector faces a growing need for applied research that allows for development of new, more attractive, and profitable forest products. One of the highest value-added products in the Mediterranean forest is the pinenut for its nutritional content as well as considered a healthy food. In addition, this edible seed is an essential ingredient in many traditional sweet recipes, "panellets" or "coca of pinenuts" in Catalonia or as a basic ingredient in sauces such as Italian "pesto" or for mediterranean sauces for meat roasts. Due to its excellent taste and cultural relevance in the Mediterranean diet, the pinenut becomes one of the most emblematic non-timber forest products of Mediterranean forests, with a market generating several hundred million euros annually (MUTKE *et al*., 2012 & 2020).

This pine, spans approximately 36,000 hectares in the Catalan territory, northeast of Spain, where is often found in combination with other species such as *Pinus halepensis*, *Quercus ilex* subsp. *ilex*, and *Quercus suber* (BELTRÁN *et al*., 2022). The Stone pine thrives at altitudes ranging from 0 to 1,000 meters, with annual water requirements between 400 and 800 mm. It is a heliophilous species that tolerates summer drought and mild frosts. The auto-ecology of this species makes it very attractive to occupy marginal rainfed agricultural land in the climatic scenario that is being drawn in the Mediterranean area (SÁNCHEZ-GÓMEZ *et al*., 2009; SBAY, 2016; ABAD VINES *et al.,* 2016; WAHBI *et al.,* 2020; GUÀRDIA *et al*., 2021).

Although the pinenut as food dates since ancient times, from an agronomic perspective, it is still considered a forest tree. Still now, the pinecone harvest comes from natural forests or naturalized stands. That is the current situation both in traditional producer countries, such as Portugal, Spain or Turkey, or in the new ones such as Chile or New Zealand. Under these conditions, the production of pinecones is so much variable, ranging annually from 285 kg/ha to 650 kg/ha in Catalonia depending on the size and lightening of the crown (PIQUÉ *et al*., 2009).

In the 1980s, extensive prospecting work was carried out in the Iberian Peninsula culminating in the selection of highly productive genotypes of *P. pinea* that are currently catalogued for use as clonal Forest Reproductive Materials (FRM) of tested category, five clones, and as qualified, ten more (MUTKE *et al*., 2007 & 2017). In parallel, it was found that a grafted genotype advances the entry into production by more than 10 years *vs* a seedling (MUTKE *et al*., 2000; GUADAÑO, *et al*., 2016; LOEWE *et al*., 2024). The fact that all grafted trees can

have a very similar productive response and to give first pinecones in the third vegetative period were the key points to start with the agronomical domestication of the species. it seems that the time has come for the development of grafted stone pine plantations.

The yields from these new plantations using grafted plants surpass those from natural forests. Although experiences are still limited, and some untested plant materials have been used, there is already data indicating that certain plots have achieved production levels exceeding 1,500 kg/ha after just eight years (DE LA MATA et al., 2019). Within the framework of the Quality Pinea project (Interreg POCTEFA 2014-2020), an evaluation of the management needs and expected production in these new plantations was made and the economic estimates with 13 years of data were very encouraging (ROVIRA, 2021).

The demand for grafted plants with the selected clones far exceeds the current supply of grafted plants in Spain. In Forestal Catalana (referred as FC), the first issue was to get these selected clones to be marketed, and this was possible in 2019, when the orchard producing scions could be registered to the Ministry of Ecological Transition of Spain. Since then, work has been done to produce grafted plants but also to help the installation of new mother fields in different locations from Spain.

Currently, the main difficulty of grafting stone pine lies in the short period of time available for grafting, as soon as the needles of the scion develop the scion is useless. This means that in a narrow window, production must be as efficient as possible.

The work over four years to achieve this objective is presented. This article delves into the various factors that influence grafting success, including the impact of Degree Days (MUTKE *et al.,* 2003) to establish the optimal grafting window, the role of clone provenance, and the management of shoot production. Analysing data from over 7,300 grafts, our aim has been to provide insights into best practices for increasing the efficiency of *Pinus pinea* grafting. As we explore the findings and implications of this research, we hope to contribute to the ongoing efforts to cultivate this valuable species.

Materials and Methods

Mother plant fields

To commercialize FRMs Spanish regulations are outlined in Royal Decree 289/2003, which incorporates the European Directive 1999/105/EC into national

law. Within this legislative framework, the stone pine grafting program of FC is being developed. This program aims to establish mother plant fields or clonal orchards to produce trees from selected clones listed in the National Catalog of Basic Materials.

The scions used during the four years of this work came from the mother plant field of FC nursery operating in Torreferrussa, located in Santa Perpètua de Mogoda, (UTM X, UTM Y 430096.00, 4597832.00) established in September 2016 and officially commissioned in 2019. This orchard meets all currently declared clones (15 in total), each individually identified by their molecular profile (MUTKE et al., 2017).

The scion producer orchard of Torreferrussa consists of 10 ramets from each declared clone, totalling 150 individuals. Seedlings of *Pinus pinea* were grafted with the different clones. These trees are arranged in a planting grid of 6x6 and cover an area of 5,500 square meters. The plant material consists of *Pinus pinea* grafted onto rootstocks of the same species.

Before planting, the plot was stumped, subsoiled and amended organically, and then tilled and milled to plant. The first three years the soil was cultivated between rows and subsequently the green cover was maintained with two mowing passes per year. The plot is managed as rainfed but has an irrigation system to provide water in the spring if it does not rain at that time of the year. The general management of the field is aimed at avoiding the effect of any external factor limiting the growth of the year. In addition to the lack of water, the characteristics of the soil, in this case heavy and alkaline, and the phytosanitary problems must also be controlled.

As of September 2024, the trees in this field have reached a height of 66 cm at the point where the crown begins, with an average diameter of 2.5 cm. The overall average height of the individual trees is 220 cm.

Figure 1. Mother plant field at Torreferrussa (Santa Perpètua de Mogoda).

Degree-Days

The scions should be collected between 460 and 760 Degree-Days (DD) following the indications of MUTKE *et al*. (2003). The optimal scion's collection phase for our location in northeastern of Spain is between the end of March and the beginning of May. For the calculation of the DD corresponding to this period of the year, the climatic data from the nearest agrometeorological station, Caldes de Montbui, located at UTM X, UTM Y 430709.00, 4607105.00, and at an altitude of 176 m a.s.l., were used.

Grafting Technique

For grafting, the terminal guide substitution technique is used, where the main guide of the rootstock is replaced by the dominant guide of the selected scion. The necessary tools for performing the graft are pruning shears, a scalpel, grafting tape, and alcohol.

For the preparation of the rootstock, all lateral guides of the last whorl were removed, leaving only the main one; lateral branches taller than the main one were cut back, then the terminal guide was cross sectioned between 1 and 2 cm above the last whorl, and a longitudinal cut was made of the same length as the wedge of the scion.

For the preparation of the herbaceous scion, it was cut obliquely on both sides with a wedge length between 2 and 3 cm.

The two plant portions were tied together with buddy tape and covered with a perforated transparent plastic bag. The bags were removed 30 days after the grafting was performed.

Figure 2a. Grafted stone pine plants.

Figure 2b. Detail of a grafted plant.

Plant material

Scions were collected on the same day, or no more than one day before the grafting was performed. They were stored in a cool place until the time of grafting. All scions came from codominant shoots of the mother plant. Scions longer than 6 cm were discarded. The annual harvest limit for each mother plant was set at 27% of the existing annual scions.

The rootstocks used were seedlings of *Pinus pinea* from the provenance region ES03-La Mancha, 2 or 3 years old, with a height between 60 and 70 cm and an average diameter of the terminal shoot at the grafting section of 8 mm.

All rootstocks were grown in forest containers with a capacity of 3.5 Liters, and they were in a vegetative state equal to or more advanced than the scions when grafted.

The 15 clones registered in Spain were used in the four years, the quantity of each one of them was in relation to the quantity of available scions, although it was very balanced (Table 1).

Table 1. Number grafts made from 2021 to 2024 per clone

PR	ID	2021	2022	2023	2024	TOTAL
Meseta Norte	1011	59	155	264	169	647
	1012	20	132	126	160	438
	1073	37	88	129	130	384
	1123	40	104	149	110	403
	1201	36	90	98	116	340
Valle del Tiétar y del Alberche	2004	63	107	225	146	541
	2048	40	130	203	116	489
	2068	68	97	163	146	474
La Mancha	3029	20	208	241	98	567
	3048	85	118	215	123	541
	3057	23	113	138	105	379
	3063	90	194	243	225	752
Catalunya Litoral	6010	20	102	83	69	274
	6015	38	125	159	138	460
	6053	80	129	279	188	676
		719	**1,892**	**2,715**	**2,039**	**7,365**

PR: Provenance Region of Spain

Grafting in nurseries

The grafts were made under greenhouse conditions, between the hours of 09:30 and 14:30 (UTC+02:00), with minimum temperatures of at least 15ºC and average relative humidity above 50%.

A total of 7,365 grafts were performed in the four years from 2021 to 2024 (Table 1). As far as possible, the grafters were the same each year and the clones were randomly distributed among them, always in scion groups of a minimum of 3 to a maximum of 30. Graft yields in the nursery were between 18 and 20 per person per hour.

Recorded data and analysis

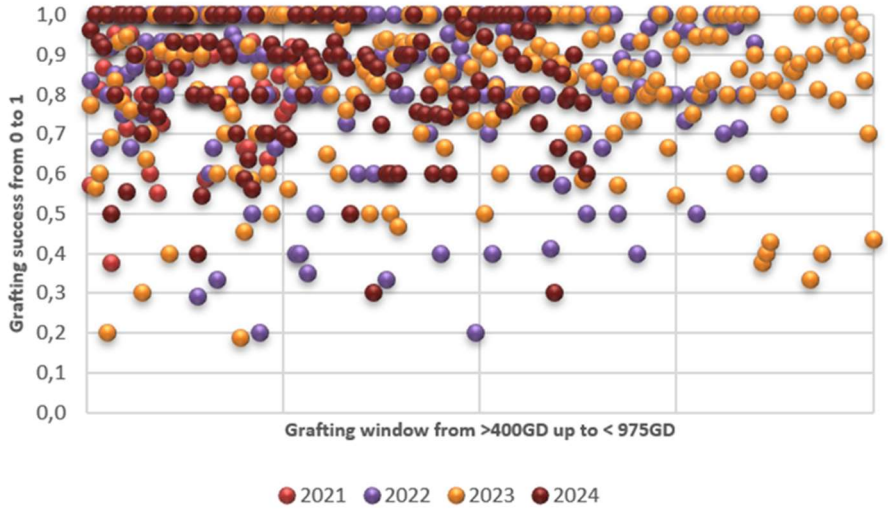
All the scions removed from the mother plants were counted globally by clone. The percentage of grafting success was evaluated by grafter, clone and day (corresponding to a DD).

The statistical analysis was made using a mixed model approach, considering year and clone as fixed factors and grafter as replications. All analysis were performed using the software SAS 9.4.

Results

Grafting Success:

The average grafting success over four years, from 2021 to 2024, was 82.6% (SD ±1 between years), based on more than 7,300 grafts. As shown in Figure 3, the range of grafting success varied from 20% in the worst cases to 100% in the best for the 15 materials propagated. No significant differences were observed between years. However, the experience of the grafter does influence success rates, but once a well-trained team is established, the level of success remains consistent.



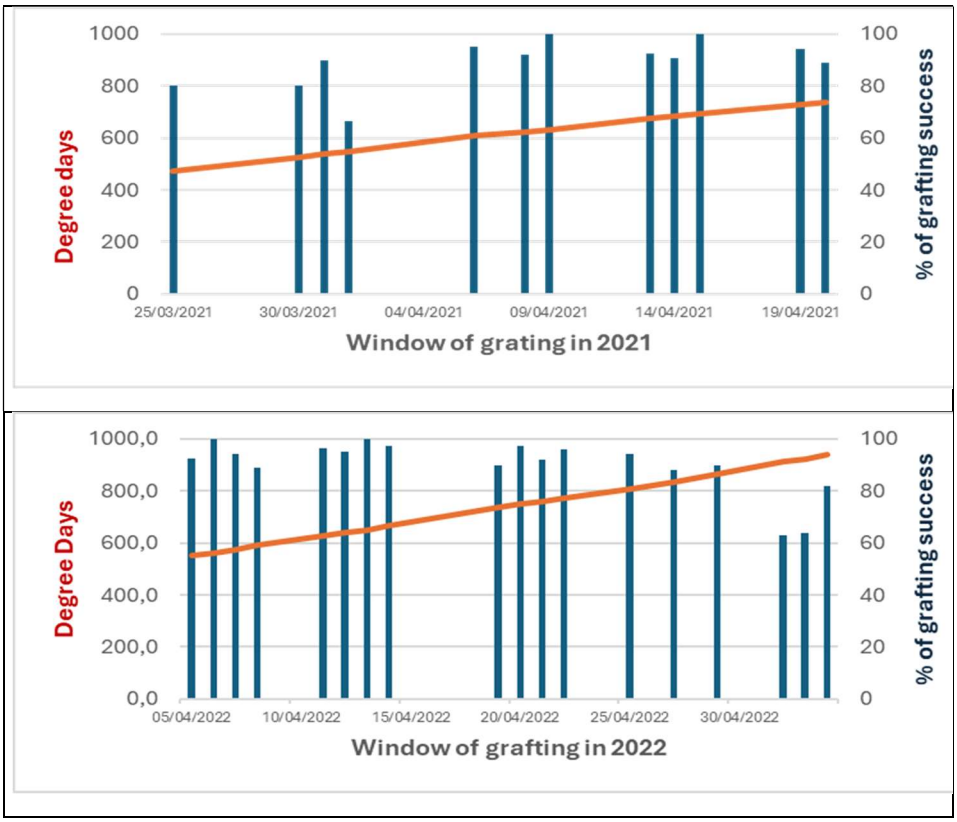
Each dot corresponds to a date and a clone. Success is measured on a minimum of 3 grafts and up to a maximum of 30 grafts, always performed on the same day & clone and by the same grafter.

Figure 3. *Pinus pinea* onto *Pinus pinea* grafting from 2021 to 2024.

Effect of Degree-Days on the grafting period:

The grafting window, determined by calculating DD at the grafting site, varies each year. In 2021, it was extended by 26 days, starting on March 25. In 2022, it expanded to a full month, beginning on April 5. In 2023, the window was 41 days, starting on March 23, and in 2024, it extended by 28 days, starting on March 21.

In Figure 4, the grafting periods for different years are summarized. In 2021, grafting began just below 500 DD and ended after 700 DD, aligning with the recommendations of Mutke *et al.* (2003), and the grafting success remained high throughout the period (719 grafts). In 2022, grafting started at around 550 DD and concluded after 900 DD, with a decline in yield beyond 900 DD (1,892 grafts). In 2023, grafting began after 400 DD and continued until after 900 DD, showing a similar trend as the previous year, with success dropping after 900 DD (2,715 grafts). In 2024, grafting commenced slightly below 500 DD and stopped at 900 DD, maintaining very high success rates throughout the period (2,039 grafts).



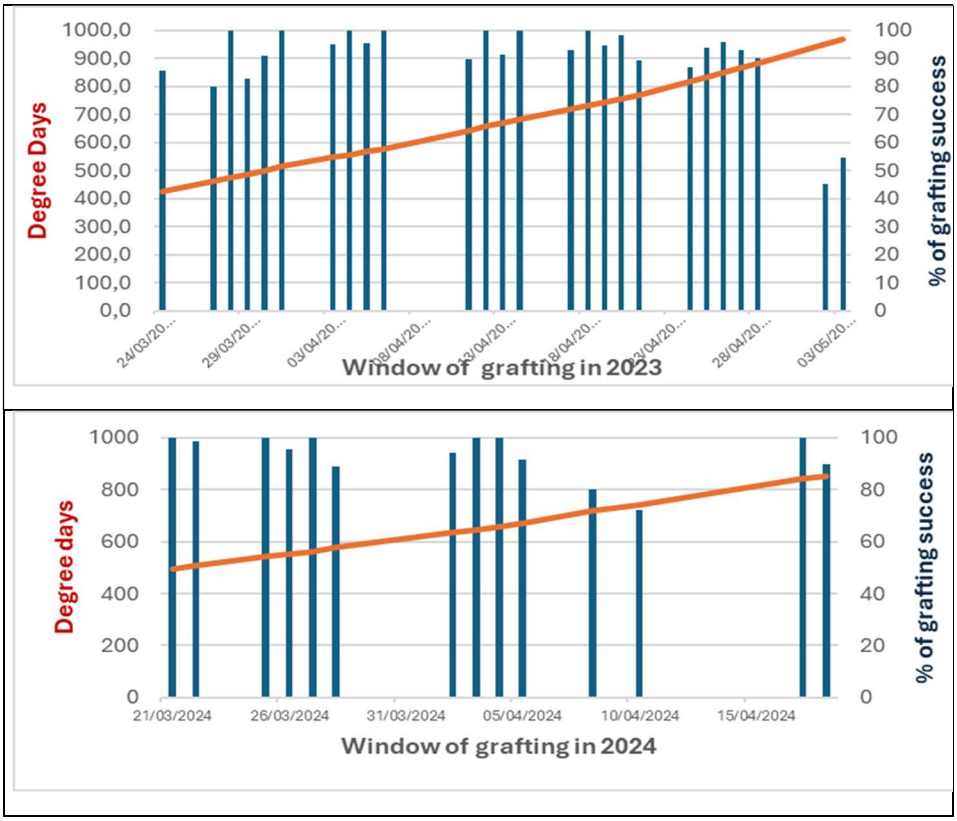
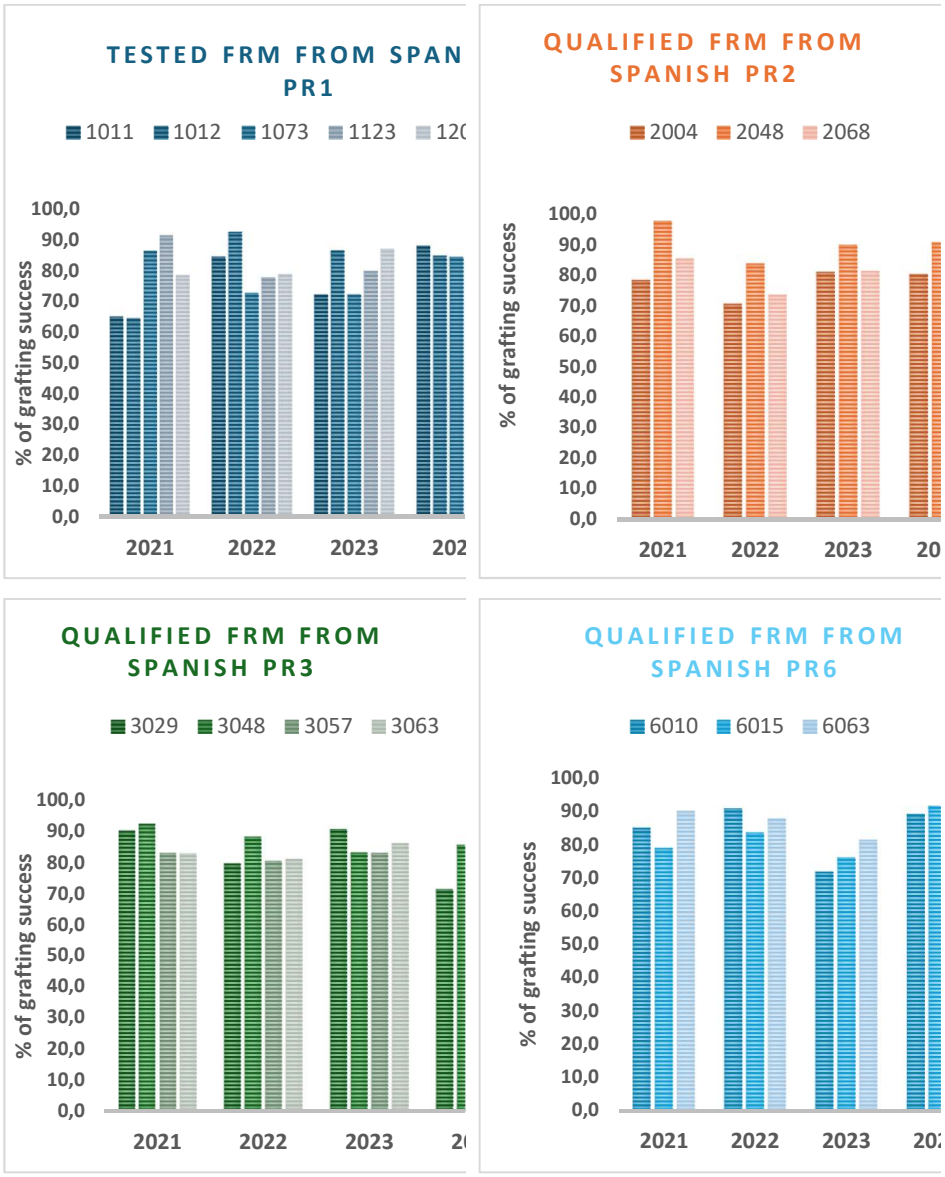


Figure 4. Grafting success *vs* Degree Days of the four years under consideration

These results suggest that, in the conditions of the FC nursery located in Santa Perpètua de Mogoda, northeast Spain, the optimal Degree-Days (DD) for successful grafting of *P. pinea* range from just over 400 to 900. This indicates a slight expansion of the recommended grafting window (MUTKE *et al.*, 2003).

Effect of clone:

In Figure 5, the grafting results are shown, categorized by provenance regions and the corresponding registered clones in Spain. So far, there are no significant differences observed between the provenance regions or between the clones.



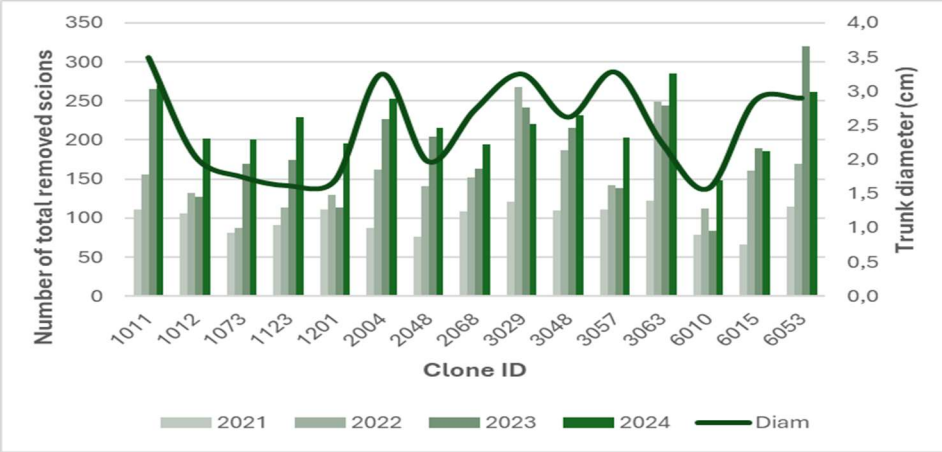
PR1: provenance region of 'Meseta Norte'; PR2: provenance region of 'Valles del Tiétar y del Alberche'; PR3: provenance region of' La Mancha' & PR6: provenance region of Catalunya Litoral.

Figure 5. Percentage of grafting success on the Spanish 15 registered clones of *P. pinea* grafted onto *P. pinea*.

Scions Produced in the Field Plot Established for This Purpose:

The availability of suitable scions for grafting is a major limiting factor in the production of grafted stone pine plants. Trees require time to produce scions in sufficient quantity, and it is essential to practice restraint in their extraction to avoid harming future production. In fact, careful management of scion harvesting can promote healthier production in subsequent years.

Forestal Catalana Orchard for Scion Production, which includes 15 *Pinus pinea* clones registered in Spain, is now 9 years old and has produced approximately 3,500 quality scions for grafting in 2024. The vegetative development of the pines since 2019, the year this *P. pinea* FRM production of this orchard was officially commissioned, is summarized in Figure 6.



Trunk diameter of 2024 measured at the canopy opening point. 10 trees per clone form this scion orchard.

Figure 6. Evolution of scion production in the scion orchard of Forestal Catalana and secondary growth of trees reached in 2024.

The plot is well managed, with trees receiving supplemental irrigation during drought periods, promoting relatively uniform growth. However, it has been observed that some clones, such as 6010 (as indicated in the graph), exhibit less vigour compared to others in the orchard and tend to have lower scion production.

By the 6th year (2021), most clones were producing around 100 scions, averaging 10 usable scions per tree. By 2024, at 9 years, most clones had doubled their production. Figure 7 illustrates the projected trend in scion production per tree based on the four years of available data. According to the data, time accounts for only 50% of the influence on the production of usable scions.

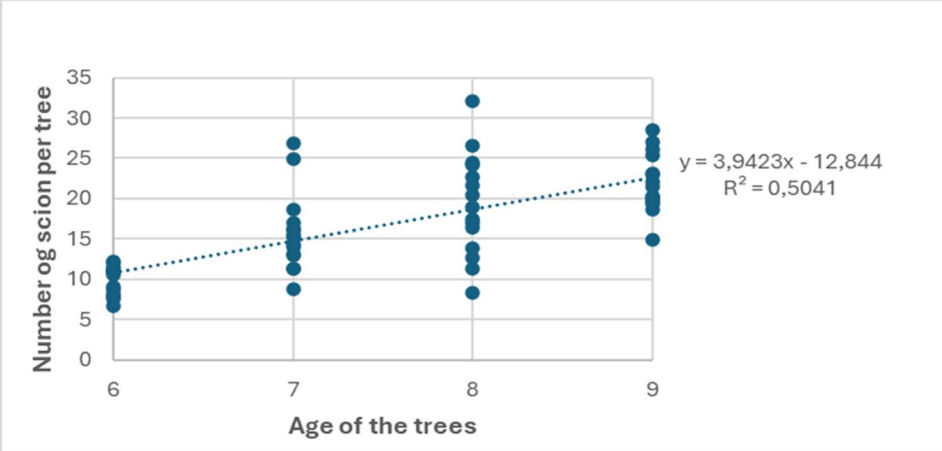


Figure 7. Evolution of scion production in the orchard of Forestal Catalana. Data of four years.

Discussion

The success rate of grafting *Pinus pinea* selected clones onto *P. pinea* rootstock has typically exceeded 80% over the past four years at the FC nursery. Although failures do occur each year, with success rates dropping below 60%, these are usually due to the grafter's inexperience, or the poor quality of scions caused by inadequate storage.

The grafting period for this species, which is naturally short, has been determined using DD as calculated by MUTKE *et al.* (2003) and this tool has proven very effective. However, it has been observed that under the conditions at the FC nursery, this grafting window can be extended to up to 900 DD, still maintaining high success rates. This finding suggests the need for further study to determine the optimal conditions for high grafting success in different regions and sites.

Regarding the grafted clones, the genotype does not appear to significantly influence grafting success, though there have not been enough years of

observation to draw definitive conclusions. Given the known plasticity of the species ((SÁNCHEZ-GÓMEZ *et al.*, 2009).), the uniformity in grafting results may be explained by the fact that all scion-producing trees are in the same plot and managed under identical conditions. Additionally, scions are consistently harvested at their optimal vegetative stage, either on the same day or no more than one day before use, ensuring their quality.

Since 2019, the production of the FC scion orchard has been evaluated annually after its components were identified using markers (microsatellites) and registered for exploitation. Over these four years, the number of harvestable scions per tree has increased from 10 to more than double. However, the production rate remains slow, and harvesting the trees is becoming increasingly difficult.

Conclusions

The current approach involves using co-dominant buds and limiting the removal of scions to only 27% of those available on each tree for grafting. While this method has been followed so far, it results in a relatively low number of scions.

Currently, the primary bottleneck in the production of grafted *Pinus pinea* is no longer the grafting process or the availability of quality scions, but rather increasing scion production from registered scion orchards. The most straightforward solution is to establish more scion production fields or expand existing ones. However, future efforts should focus on optimizing the management of trees dedicated solely to scion production. This includes the application of appropriate training and pruning techniques, avoiding in any case the dual use of trees for the production of pinecones and scions. It would be necessary to adopt agronomic practices/management to ensure that the scions reach the ideal size for grafting according to the typical diameter of *P. pinea* rootstocks of 2 to 3 years.

Extending the grafting period is also a new objective, although it appears challenging to achieve. One potential improvement could be leveraging the fact that the nursery operates scion orchards in different climatic conditions. This allows scion production from orchards located in warm areas to be followed by that of the other in colder sites, effectively lengthening the overall grafting season.

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