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Effect of pretreatment on pecan nut germination and rootstock production

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Abstract

Pecan [*Carya illinoinensis* (Wangenh.) K. Koch] is a deciduous tree of the Juglandaceae family, native to temperate regions and growing wild in North America. In Italy, pecan trees are cultivated mainly in the southern regions, where the climatic conditions are optimal for their development. However, the area under cultivation is limited and most of the nuts are imported from the USA and Mexico. A major limiting factor for Italian pecan cultivation is the scarcity of rootstocks suitable for local pedoclimatic conditions. To evaluate the effect of stratification on the germination of pecan seeds to obtain rootstocks, seeds of 5 different cultivars were stratified and compared with those not stratified. The results indicated significant differences in nut germination related to stratification treatment and cultivar. In general, stratification process reduced the germination time from on average 50.4 days for untreated seeds to 12.6 days for treated seeds. The Wichita cultivar exhibited the highest germination rate under stratification whereas the lowest values were observed in non-stratified Cape Fare seeds. On average, better seedling development was observed in the first 6 weeks with stratification, while non-stratified nuts showed better seedling development between week 7 and 11. Shawnee and Wichita cultivars particularly benefited from the treatments, showing superior growth metrics values (35.0 ± 7.9 and 34.9 ± 7.8 cm height; 0.5 ± 0.8 and 0.5 ± 0.6 cm diameter, respectively). Cold stratification produced uniform and robust plants, providing nurseries with quality grafting material. Seeds stored at room temperature for two seasons completely lost their viability, rendering them unusable.

Keywords: *Carya illinoinensis*, dormancy removal, germination, growth rate, seed treatment, stratification

1 Introduction

The pecan tree [*Carya illinoinensis* (Wangenh.) K. Koch] is a deciduous tree of the Juglandaceae family, found mainly in temperate regions. Indigenous to North America, pecan trees thrive in various pedoclimatic zones but show a preference for warmer climates due to their extended growing season. Commercial cultivation of pecan is primarily concentrated in the southern United States, Brazil, Australia, Canada, South Africa, and Israel (Casales *et al.*, 2018). In Italy, however, pecan cultivation is limited and mostly ornamental, particularly in regions such as Sicily. Despite Italy's favourable soil characteristics and climatic conditions, which could support pecan cultivation with appropriate irrigation practices (Hend *et al.*, 2016; Ferrara *et al.*, 2023), only 33 hectares are currently dedicated to pecan cultivation (ISTAT, 2023).

Globally, pecan production was approximately 139,739 tonnes in the 2019/2020 crop year (INC, 2019-2020). Mexico and the USA are the largest producers, accounting for 47 % and 43 % of the total production, respectively, followed by South Africa, Australia, and Brazil. Recently, South Africa has expanded its pecan cultivation area, increasing national production by about 5 % (INC, 2019-2020). The primary exporters are Mexico and the USA, with Mexico mainly exporting to the USA. Europe, including the UK, and Canada are the major importers (INC, 2019-2020). In Italy, all pecans consumed are imported, with a trade value of around 2.5 million euros in 2018 (Eurostat, 2018).

A significant barrier to expanding pecan cultivation in Italy is the lack of rootstocks tailored to local pedoclimatic conditions. Additionally, the lengthy propagation phase

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(Zhang *et al*., 2015), especially from seedlings, poses challenges. Pecan seeds often exhibit natural dormancy from 4 to 6 months before germination, and their germination can be unpredictable and variable depending on environmental conditions. This complicates the production of sufficient rootstocks from seeds, prompting the use of alternative propagation techniques such as cuttings or layering. According to Casales *et al*. (2018), vegetative propagation methods like cuttings or layering require precision and time to produce high-quality rootstocks with a high rooting rate.

The production of pecan rootstocks typically involves grafting commercial cultivars onto seedlings derived from wild plants, which are prone to irregular or reduced germination. Failures in rootstock production are often due to the extended period required to obtain graftable plants (Ferreira da Silva *et al*. 2023). Additionally, seed moisture loss over time can significantly reduce viability (Wazir, 1976).

Dormancy in pecan seeds has been a subject of extensive research. Adams & Thielges (1978) and Ghazaeian *et al*. (2012) suggested overcoming dormancy by storing seeds at 3 to 7 °C. Stratification at higher temperatures, such as 32 and 30 °C, has also been shown to improve germination speed (Smith *et al*., 1997; Bustamante *et al*., 2006). Reid (2011) described a straightforward cold stratification method for removing pecan seed dormancy on his blog. More recently, Liu *et al*. (2022) attempted to enhance germination and seedling emergence by treating the endocarp surface.

Based on these findings, this study aimed to evaluate a simple, cost-effective cold stratification method to produce viable pecan seedlings for rootstock development in Italy. Additionally, the study assessed the impact of seed treatment on germination percentage, germination rate, and seedling development across different pecan cultivars. The viability of seeds stored at room temperature for two years was also examined by subjecting them to the same stratification treatment as fresh seeds.

2 Materials and methods

2.1 Experimental design

This study utilised five pecan genotypes selected from 40 varieties within the FAO RGV project (2020-22) and cultivated at the Council for Agricultural Research and Economics (CREA) – Research Center for Olive, Fruit, and Citrus Crops in Central Italy. The research was conducted over the 2020-2022 crop years. The chosen genotypes included four commercial varieties (Wichita, Stuart, Cape Fear, Shawnee) and one wild genotype used as a control. These genotypes were selected for their uniform and vigorous vegetative growth, high production, and adaptability to the temperate climate of Central Italy. Seeds were harvested in November of each year and stored at room temperature until use. Dry seeds harvested in November 2020 were used in 2021, while those harvested in November 2021 were used in both 2022 and 2023. To ensure a sufficient number of seeds to be used, more than 150 seeds were collected for each genotype in both year 2021 and 2022. Before being used, nuts were screened for uniform appearance and absence of defects or decay. All selected seeds were weighed and divided into weight classes. To avoid possible failures in seed germination, determined by empty seed, only the nuts from the higher weight classes were used.

A total of 240 nuts (48 seeds for each genotype) were used in each year.

2.1.1 Cold stratification process (thesis 1)

The first set of 120 nuts (24 for each genotype) were subjected to cold stratification (thesis 1). The seeds of each genotype' were immersed in cold water at 7 ± 2 °C for 24 hours. The seeds were then layered in coconut peat with field capacity moisture and completely covered (Fig. 1a and 1b). The peat and seeds were then placed in thermal containers with lids to maintain a constant temperature and humidity and stored in a refrigerator at 7 ± 2 °C for 90 days.

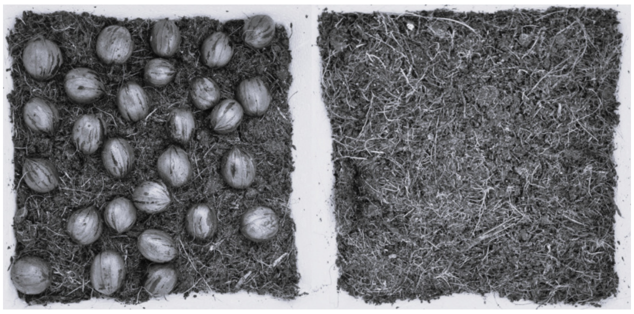

Fig. 1: *Seed samples stratification: (left) before covering, (right) after covering.*

The duration and optimal conditions for stratification were deducted from empirical tests carried out in the years preceding 2020. After the 90-day stratification period, the seeds were extracted from the coconut peat and transferred into individual plastic pots (8 × 8 × 10 cm). The substrate in the pots consisted of 80 % Brill VMG potting soil (pH 6.0, electrical conductivity 0.55 dS/mc, total porosity 90 % v/v, dry bulk density 180 kg/mc) sieved with an iron mesh with 8 × 8 mm holes, and 20 % potting soil for planting and cuttings (composed of Irish black peats, Baltic blond peats, and river silica, with 20 % dry weight of organic carbon, 0.5 % dry weight of organic nitrogen, 40 % dry weight of organic matter, and 39.5 % inert material).

2.1.2 Non-stratified seeds (thesis 2)

The second set of 120 seeds were not-stratified (thesis 2). These seeds were stored at room temperature in paper bags at 15 ± 3 °C and 80 ± 2 % relative humidity without any further treatment until sowing.

2.1.3 Seed sowing and growth conditions

All seeds (stratified and non-stratified) were sown in the centre of the pots at a depth of approximately 2-3 cm, depending on seed size, to ensure full seed coverage. The pots were then thoroughly watered to compact the soil around the seeds and then transferred to a cold glass greenhouse until seed emergence. To minimise potential effects of lighting, temperature, air currents, and edge effects on plant growth, the pots were randomly arranged on the bench. After germination, the seedlings were transferred outdoors under a shade net equipped with automatic sprinkler irrigation system with air conditioning functions.

2.1.4 Agronomic practices and monitoring

All samples were subjected to identical agronomic practices, including irrigation, temperature, and humidity management, and were monitored using data logger sensors. Plant pests and diseases were monitored, and phytosanitary treatments were applied as necessary. The protocol described remained consistent across all stratification trials conducted in 2021, 2022 and 2023.

2.2 Seeds germination evaluation

To assess the effects of the treatments on seed germination and seedling development, several key parameters were measured:

1. Emergence time: This was defined as the number of days after sowing until the seedling was no longer dependent on non-renewable seed reserves. Emergence time was recorded for each seedling to track the duration needed for each to reach a self-sustaining stage.
2. Number of germinated seeds: The total number of seeds that successfully germinated in each treatment group was recorded. Germination was considered successful when the seedling had emerged from the soil.
3. Seedling height: The height of each seedling was measured weekly using a stick meter from the first emergence (T1) to the eleventh week (T11). Measurements were taken in millimetres, and the weekly growth increments were noted.
4. Number of seedling diebacks: Instances of seedling mortality or failure to thrive were recorded throughout the trial period. Dieback was noted whenever a seedling ceased to grow or visibly declined in health.
5. Stem collar diameter: At the conclusion of the trial, the diameter of the stem at the base (collar) of each seedling was measured using a digital calliper. This measurement provided an indication of the seedling's overall robustness and structural integrity.
6. Germination percentage and rate: The germination percentage was calculated as the ratio of germinated seeds to the total number of seeds sown, expressed as a percentage. Germination rate, following Maguire (1962), was computed to reflect the speed of germination across different treatments.

Seedling growth was monitored weekly from the first emergence after sowing (T1) to the eleventh week after sowing (T11). This included recording any failures due to lack of germination or subsequent seedling death. At the end of the trial (14 weeks after sowing), final measurements of stem collar diameter and seedling height were taken, and the number of non-germinated or dead seedlings was recorded in accordance with the methodology of Poletto *et al.* (2015).

2.3 Statistical analyses

The collected data were statistically analysed as follows:

1. Data expression and normality: Results were expressed as mean ± standard deviation (SD). The normality of the data distribution was assessed using the Shapiro-Wilk test.
2. Covariance equality: The Box's M test was used to evaluate the equality of covariance matrices, ensuring that the assumption of homogeneity was met.
3. Handling missing data: Any missing data points were replaced with the mean plus two standard deviations, which helped mitigate the impact of outliers on the analysis.
4. Analysis of variance (ANOVA): A two-way ANOVA was conducted to determine significant differences between treatment groups. Tukey's post hoc test was used to identify specific differences between sample means.
5. Principal component analysis (PCA): PCA was employed to examine the vegetative behavior of the different pecan cultivars in relation to the two treatments. This multivariate technique helped identify patterns and potential groupings of samples based on similar properties.

All statistical analyses were performed using Past software (version 4.2; Hammer *et al*., 2001), which provided comprehensive tools for managing and interpreting the experimental data.

3 Results

3.1 *Effect of treatment on seeding germination*

Since the results obtained from the stratification trials conducted in 2021 and 2022 were substantially overlapping, all reported data represent the average of values recorded over these two years of testing.

3.1.1 *Germination outcomes and timing*

The influence of the stratification process on the seed germination of each genotype is detailed in Table 1. On average, the first seedling emergence for stratified seeds occurred 20 days after sowing across all investigated genotypes. Specifically, the cultivar Stuart showed the maximum number of germinated seedlings at 20 days, after which no further germination was observed. For the Cape Fear cultivar, germination was completed 27 days after sowing, whereas for the Shawnee cultivar and control samples (wild genotype), germination concluded 34 days after sowing. Wichita exhibited germination until 48 days after sowing.

In contrast, non-stratified seeds exhibited a delayed germination onset, with the first seedling emergence varying among cultivars. The first seedlings to emerge were from the Cape Fear and Shawnee cultivars (27 days after sowing), followed by Stuart, Wichita, and Wild (34 days after sowing). The duration of the germination process for non-stratified seeds extended significantly longer than that for treated seeds: 69 days for the Cape Fear and Wild samples and 90 days for the Stuart, Shawnee, and Wichita cultivars (Table 1).

3.1.2 *Germination rate and seedling viability*

The total number of seedlings emerged was not significantly influenced by the treatment but rather by the genotypes. The Cape Fear cultivar exhibited the lowest number of emerged seedlings. In contrast, Shawnee and Wichita cultivars showed the highest numbers of emerged seedlings. For the other genotypes, the number of seedlings emerged ranged between 22 and 23 days (Table 1). Figure 2 illustrates the different germination timings and rates registered for the different genotypes in relation to the treatment. Generally, the stratification treatment significantly improved the speed of germination but did not markedly affect the final germination count or seedling viability.

Fig. 2: *Delay in germination based on different treatment. On the right stratified seeds; on the left non-stratified seeds at 30 days after sowing.*

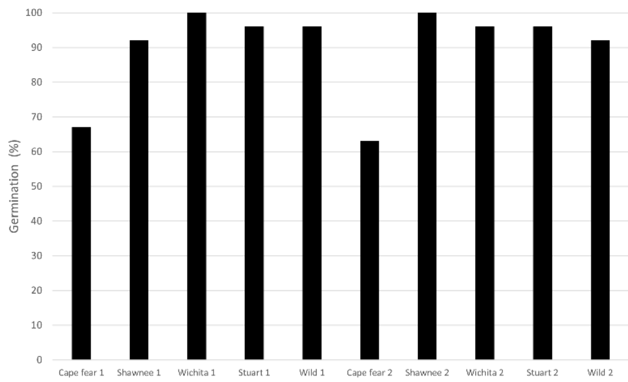

Fig. 3: *Germination percentage of seeds of five different cultivars and two treatments. Number "1" denotes stratified seeds; number "2" non-stratified seeds. Average of the values recorded in 2021 and 2022.*

3.1.3 *Germination percentage*

Regarding the germination percentage, the best results were obtained by the cultivars Shawnee/thesis 2 and Wichita/thesis 1. Hight germination percentage were also reported for the genotype Wichita/thesis 2, Stuart (both treatment), and Wild/thesis 1. Good percentage was obtained for Shawnee/thesis 1. The worst results were observed in the cv Cape Fear whose seeds had the lowest germination percentage (Fig. 3).

3.1.4 *Germination rate*

The germination rate was highest for Wichita/stratified (Wichita 1), followed closely by Stuart/stratified (Stuart 1). Cape Fear/non-stratified (Cape Fear 2) had the lowest germination rate. The germination rates for other genotypes and treatments were variable, with a general trend indicating

Table 1: *Germination outcomes in the two theses. Average of values recorded in the years 2021 and 2022 (n=24 per treatment).*

Cultivar	Germination duration from sowing (days)		Total number of emerged seedlings		Dead seedlings		Plantlets with shoots		Germination percentage (%)	
	1	2	1	2	1	2	1	2	1	2
Cape Fear	20-27	27-69	16	15	5	1	3	1	67	63
Shawnee	20-34	27-90	22	24	3	1	3	0	92	100
Wichita	20-48	34-90	24	23	1	1	1	0	100	96
Stuart	20	34-90	23	23	5	1	3	1	96	96
Wild	20-34	34-69	23	22	3	0	3	0	96	92

1 = stratified; 2 = non-stratified

higher rates for stratified seeds compared to non-stratified ones (Fig. 4). The wild genotype showed a low germination rate for both treatments.

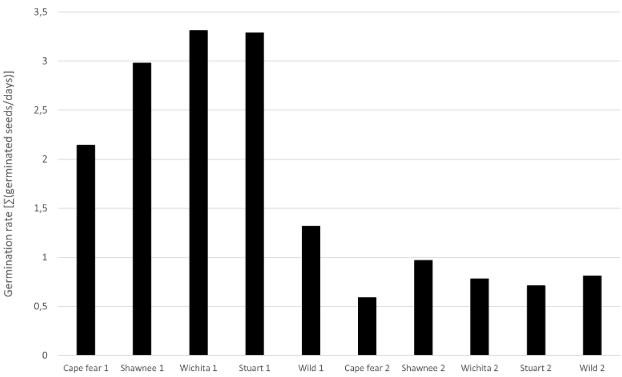

Fig. 4: *Germination rate of seeds of five different cultivars and two treatments. Number "1" denotes stratified seeds; number "2" non-stratified seeds. Average of the values recorded in 2021 and 2022.*

During seedling growth, failures of unknown cause occurred, resulting in delayed development or death of the apical part of the seedlings. In the latter case, the seedlings were generally found to be viable and ready to shoot from the basal part of the collar with complete replacement of the desiccated part.

3.2 *Effects of treatment on seedling development*

The seedlings derived from the two different treatments showed clear differences in development, as shown in Tables 2 and 3. The analysis of variance (ANOVA) revealed significant differences between genotypes, treatments, and their interaction concerning height and diameter. The treatment was identified as the primary factor influencing both parameters, while the interaction between cultivar and treatment had a lesser impact.

Table 3 shows the results of seedling development at the end of the test (14 weeks after sowing). Regarding height and diameter, the cultivars Shawnee and Wichita showed the greatest height and diameter at the seedling collar. The Stuart cultivar showed the lowest height values (18.69 ± 5.20 cm), and the Cape Fear cultivar the lowest diametrical growth (0.332 ± 1.03 cm). Referring to the height/diameter ratio (H/D ratio), this ranged from a minimum of 6.08 (Cape Fear) to a maximum of 7.34 (Shawnee). There was a wide intra-genotype variability in the H/D ratio, indicating differences in growth patterns among individual seedlings within the same genotype.

Table 2: *ANOVA results showing the average of the values recorded in 2021 and 2022.*

Source	*DF*	*height*	*diameter*	*H/D ratio*
Cultivar (C)	4	516.43^{***}	9.78^{***}	3.87^{ns}
Treatment (T)	1	2128.85^{***}	25.22^{***}	5.82^{ns}
CxT	4	210.24^{**}	4.29^{***}	2.46^{ns}
Whitin	230	69.37^{ns}	0.82^{ns}	2.52^{ns}
Total	239			

***: significant at $p \leq 0.001$; **: significant at $p \leq 0.01$; ns: not significant.

H/D ratio was not significantly affected by either genotype or treatment, suggesting that the proportional growth of height and diameter was consistent across different conditions and cultivars. The cultivars Shawnee and Wichita were the best performers in terms of height and diameter, while Cape Fear and Stuart showed lower values in these parameters. These findings indicated that the stratification treatment significantly influences seedling development, with notable differences observed between cultivars. The cultivars Shawnee and Wichita particularly benefited from the treatments, showing superior growth metrics.

3.3 *Principal component analyses*

To identify the vegetative behaviour of the different cultivars subjected to the two treatments, a PCA analysis was

Table 3: *Final relief of seedling development at 14 weeks after sowing. Average of the values recorded in 2021 and 2022.*

	Av. height (cm)		Av. diameter at the collar (cm)		Height-to-diameter ratio	
Cultivar	1	2	1	2	1	2
Cape Fear	26.9 ± 7.7^{Ba}	21.3 ± 9.5^{Ba}	0.36 ± 0.6^{Ca}	0.33 ± 1.0^{Ca}	7.3 ± 1.6^{ns}	6.1 ± 1.6^{ns}
Shawnee	35.0 ± 7.9^{Aa}	27.6 ± 9.8^{Bb}	0.51 ± 0.8^{Aa}	0.40 ± 1.1^{Bb}	6.9 ± 1.6^{ns}	6.9 ± 1.6^{ns}
Wichita	34.9 ± 7.8^{Aa}	24.9 ± 9.2^{Bb}	0.49 ± 0.6^{Aa}	0.40 ± 1.0^{Bb}	7.1 ± 1.6^{ns}	6.4 ± 1.6^{ns}
Stuart	27.9 ± 7.2^{Ba}	18.7 ± 5.2^{Cb}	0.41 ± 0.7^{Ba}	0.45 ± 0.7^{Ba}	6.7 ± 1.3^{ns}	6.2 ± 1.8^{ns}
Wild	28.6 ± 9.3^{Ba}	26.4 ± 7.5^{Ba}	0.45 ± 1.1^{Ba}	0.45 ± 0.9^{Ba}	6.2 ± 1.5^{ns}	6.5 ± 1.7^{ns}

Av.: average. Different letters identify significant differences ($p \leq 0.05$). Capital letters identify significant differences between varieties. Lowercase letters identify significant differences within variety in relation to treatment. 1= stratified 2= non-stratified.

performed on seedling growth throughout the trial. Figure 5 shows a clear distinction between treated (stratified) and untreated (non-stratified) samples. The stratified samples showed an early positive growth correlation early (T1–T7), while the untreated samples caught up later (T8–T11). The biplot of PC1 vs. PC2 in Figure 5 shows the "scores" for the different varieties related to seedling growth under each treatment, along with the "loadings", where each loading vector represents one of the sampling times. This also provides information on their correlation.

PC1 accounts for 77.7 % of the total variance and is positively associated with all sampling times (T1-T11). While PC2 accounts for 12.2 % of the total variance and is positively associated with times from T1 to T7 and negatively associated with times from T8 to T11.

The chemometric analysis grouped the samples into two main clusters relative to the positive and negative sides of PC1. A negative PC1 quadrant containing predominantly all non-stratified samples, and a positive PC1 quadrant containing predominantly all stratified samples.

Further classification relative to PC2 showed that non-stratified samples were positively associated with longer sampling times (T8–T11). This meant that the treatment had a positive effect on seedling development, especially in the early stages (T1–T7). The non-stratified seedlings showed a higher growth rate from T8 onwards.

3.4 *Effects of treatment on seedling growth*

ANOVA analysis confirmed significant differences ($p \leq 0.001$) in seedling growth in relation to treatment (stratification vs. non-stratification), genotypes, and sampling times. Figure 6 illustrates the trends in seedling development over time for all varieties under the two treatments.

At T1, which was 20 days after sowing, a high percentage of seeds from thesis 1 were found to have germinated. In thesis 1 (stratified seeds), two distinct groups were observed.

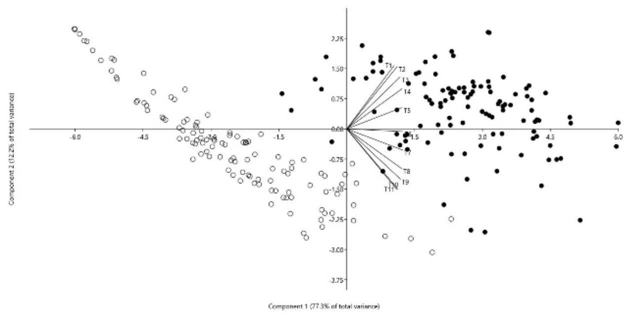

Fig. 5: *Principal component analysis (PCA) developed on plant growth data over time (T0 - T11). Average of the values recorded in 2021 and 2022.*

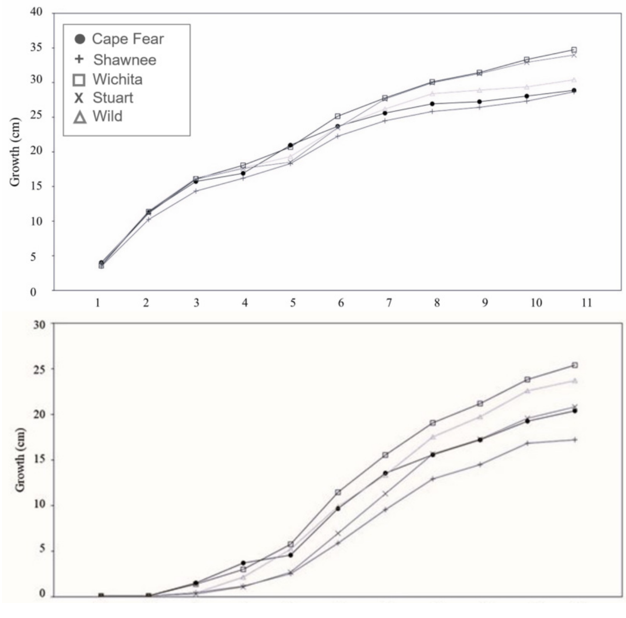

Fig. 6: *Growth trend of seedlings from stratified seeds (above), and from non-stratified seeds (under). Average of the values recorded in 2021 and 2022.*

The more vigorous group included the cultivars Wichita and Stuart, while the weaker group consisted of the cvs Shawnee and Cape Fear. The wild cv showed a development between these two groups.

In thesis 2 (non-stratified seeds), germination was more spread out between T2 and T3. The cv Wichita was the most vigorous, while the Shawnee cv was the weakest. Cultivar Stuart suffered in the absence of stratification, while Wild and Cape Fear cvs were positively influenced by stratification. Stratified seeds showed earlier and more consistent growth across all cultivars, whereas non-stratified seeds exhibited delayed and varied growth patterns. This highlights the benefit of stratification for promoting uniform and robust seedling development.

Stratification was applied to seeds that had been preserved for two years. Seeds collected in 2021 and used in 2023, after two years of storage at room temperature, were completely devitalized. None of the 240 seeds, whether stratified or non-stratified, germinated. After 90 days from sowing, visual analysis of the cotyledons and seedlings showed that 30 % of the cotyledons were water-soaked, and 70 % were moldy.

The environmental conditions during the trials were based on an average of the values recorded in 2021 and 2022. The minimum temperatures remained above 15 °C for almost two months, while the maximum temperatures stayed above 25 °C for nearly the entire trial, with a peak at 45 °C. Air conditioning irrigation in the shade house kept ambient humidity generally above 60 %, with peaks near 85 %. Consistently high temperatures and adequate humidity levels supported the test conditions, although the extreme temperatures may have affected seedling viability and growth patterns.

4 Discussion

Pecan nuts are widely valued for their taste and nutritional benefits, however Italian production remains insufficient to meet the growing market demand. Italy continues to rely on pecan imports due to the limited availability of rootstocks and the challenges associated with seedling propagation. One of the main constraints is the absence of rootstocks specifically adapted to the diverse Italian climates. Additionally, the long propagation phase, especially when using seedlings, makes commercial cultivation more complex and time-consuming. To support domestic production, efforts should focus on optimizing vegetative propagation techniques and improving orchard management practices. Cost-effective methods, such as seed stratification, could enhance propagation success and promote uniform seedling development.

This study evaluated the effect of seed stratification on five pecan genotypes. The results confirmed that stratification accelerated and synchronized germination, improving germination rates and reducing the overall germination period. Specifically, stratified seeds germinated in an average of 12.6 days, whereas non-stratified seeds required 50.4 days. These findings align with previous studies on pecan stratification (Smith *et al.*, 1997; Ghazaeian *et al.*, 2012; Poletto *et al.*, 2015) and may be linked to increased cytokinin and gibberellin levels in the embryo during stratification, which facilitate food reserve digestion and embryo growth (Dimalla *et al.*, 1978).

Despite this, germination percentages exceeded 90 % for all genotypes, regardless of stratification, except for cv Cape Fear, which exhibited lower germination rates. This high viability was likely influenced by seed weight selection, which effectively eliminated empty or non-viable seeds. Among cultivars, Wichita and Shawnee, which had the largest seeds (unpublished data), displayed the highest germination rates and final seedling size.

Seedling resilience was notable, with most plants recovering quickly from partial or complete foliage loss. However, Cape Fear and Stuart cvs showed a 20 % failure rate, suggesting they are less suitable as seedlings. Statistical analyses and PCA biplot confirmed that stratified seeds germinated approximately one week earlier than non-stratified ones, with the resulting seedlings growing at a faster rate.

Growth analysis during the T1–T11 period revealed that seedlings from stratified seeds exhibited more rapid and uniform development compared to non-stratified ones. This is likely due to the synchronised germination of stratified seeds (T1–T2), whereas non-stratified seeds germinated progressively over time (T1–T8). As a result, non-stratified seedlings showed greater variability in size and uniformity. The ratio between collar diameter and plant height was not statistically significant, as these parameters are closely related.

The substrate used in this trial effectively maintained seedling moisture and is commercially available, facilitating nursery operations. To streamline the process, pre-mixed potting soil is recommended. Furthermore, given the initial leaf burn observed in the cold greenhouse, the germination phase should ideally occur in an outdoor structure equipped with a shade net. A technologically advanced greenhouse with a shading system could further optimize seedling development.

In conclusion, cold stratification significantly improved seedling uniformity and vigor, providing high-quality material for grafting. The treatment reduced germination time and enhanced seedling development, particularly during the first six weeks. While non-stratified seedlings exhibited increased growth between the 7^{th} and 11^{th} weeks, Shawnee and Wichita benefitted the most from stratification, displaying superior growth metrics. Additionally, seeds stored for two years at room temperature failed to germinate, rendering them unusable (Wazir, 1976).

Further research is needed to validate these findings and assess the long-term performance of the best-performing cultivars.

Conflict of interest

The authors declare that they have no conflict of interest.

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