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*Artículos científicos*

## **Estratificación e incubación: ¿imprescindibles para la germinación de la nuez pecán?**

***Stratification and Incubation: Essential for Pecan Germination?***

***Estratificação e incubação: essenciais para a germinação de noz-pecã?***

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### **Resumen**

Las nueces de pecán (*Carya illinoensis* L.) germinan de manera heterogénea y en bajos porcentajes pero, al aplicar tratamientos pregerminativos, como la imbibición, la estratificación, la incubación y la aplicación de hormonas, se puede homogeneizar el ritmo de germinación e incrementar el porcentaje de semillas germinadas. Tomando en cuenta los resultados de investigaciones previas, se requiere comparar el efecto de la estratificación desnuda contra el efecto de la estratificación húmeda en nueces de nogal pecanero para determinar un tratamiento pregerminativo óptimo. El objetivo de esta investigación fue evaluar la germinación inducida mediante estratificación desnuda y húmeda en pacanas maduras. Se aplicaron tres tratamientos pregerminativos a semillas de los cultivares Wichita



y Western Schley, a saber: estratificación desnuda (estratificación a 4 °C por un mes, seguida por incubación a 30 °C durante 17 días), estratificación húmeda (estratificación a 4 °C con suministro de riego, por un mes, sucedida por incubación a 30 °C durante 17 días) y un control (estratificación desnuda a 4 °C por un mes, seguida por siembra en invernadero). Aquí mostramos que la estratificación desnuda, seguida por incubación a 30 °C, puede tener un efecto benéfico significativo sobre el porcentaje de germinación, longitud de brote y raíz, así como la disminución de la incidencia de infecciones microbianas en nueces de nogal pecanero. El efecto positivo de la incubación sobre la germinación de la nuez pecán observado en esta investigación concuerda con los resultados de trabajos previos. Se observó que el porcentaje de germinación de las nueces del cv. Wichita fue significativamente superior al aplicar la estratificación desnuda en comparación con lo obtenido al emplear la estratificación húmeda. Los resultados implican que muchas semillas de pecanero pueden prescindir de la incubación para completar el proceso de germinación, sin embargo, el porcentaje de nueces del cv. Wichita que germinaron fue seis veces superior al incluir una etapa de incubación en el tratamiento pregerminativo. En el caso de las nueces del cv. Western Schley, el porcentaje de germinación fue 28 veces más elevado en las nueces incubadas que en las nueces que no fueron incubadas. Por otra parte, el presente estudio fue incapaz de probar la prescindencia de la estratificación para inducir la germinación de las nueces de pecán, ya que todas las nueces utilizadas en este experimento fueron almacenadas en refrigeración antes de ser sometidas a los diferentes tratamientos.

**Palabras clave:** *Carya illinoensis*, semilla, Western Schley, Wichita.

## Abstract

Pecans (*Carya illinoensis* L.) germinate heterogeneously and in low percentages, but by applying pre-germination treatments, such as imbibition, stratification, incubation and the application of hormones, the rate of germination can be homogenized and the percentage of germinated seeds can be increased. Taking into account the results of previous investigations, it is necessary to compare the effect of stratification without media against the effect of wet stratification in pecans to determine an optimal pre-germination treatment. The objective of this investigation was to evaluate germination induced by bare and wet stratification in mature pecans. Three pre-germination treatments were applied to seeds of cultivars Wichita and Western Schley, namely: stratification without media (stratification at 4 °C for one month, followed by incubation at 30 °C for 17 days), wet stratification (stratification at 4 °C with irrigation supply, for one month, followed by incubation at 30 °C for 17 days) and a control (naked stratification at 4 °C for one month, followed by greenhouse seeding). Here we show that stratification without media, followed by incubation at 30°C, can have a significant beneficial effect on germination percentage, shoot and root length, as well as decreasing the incidence of microbial infections in pecans. The positive effect of incubation on pecan germination observed in this research agrees with the results of previous works. It was observed that the germination percentage of the pecans of cv. Wichita was significantly superior to bare stratification compared to wet stratification. The results imply that many pecan seeds can prescind from incubation to complete the germination process, however, the percentage of pecans of cv. Wichita that germinated was six times superior when including an incubation stage in the pre-germination treatment. In the case of pecans of cv. Western Schley, the percentage of germination was 28 times higher in the incubated seeds, in comparison with pecans that were not incubated. On the other hand, the present study was unable to prove the need of stratification in inducing germination of pecans, since all the pecans used in this experiment were stored in refrigeration before being subjected to the different treatments.

**Keywords:** *Carya illinoensis*, seed, Western Schley, Wichita.

## Resumo

A noz-pecã (*Carya illinoensis* L.) germina de forma heterogênea e em baixas porcentagens, mas com a aplicação de tratamentos pré-germinativos, como embebição, estratificação, incubação e aplicação de hormônios, a taxa de germinação pode ser homogeneizada e a taxa de germinação aumentada. porcentagem de sementes germinadas. Levando em conta os resultados de investigações anteriores, é necessário comparar o efeito da estratificação nua com o efeito da estratificação úmida em nozes pecan para determinar um tratamento ideal de pré-germinação. O objetivo desta investigação foi avaliar a germinação induzida por estratificação nua e úmida em nozes maduras. Três tratamentos de pré-germinação foram aplicados às sementes das cultivares Wichita e Western Schley, a saber: estratificação nua (estratificação a 4°C por um mês, seguida de incubação a 30°C por 17 dias), estratificação úmida (estratificação a 4°C com irrigação, por um mês, seguida de incubação a 30°C por 17 dias) e uma testemunha (estratificação nua a 4°C por um mês, seguida de sementeira em casa de vegetação). Aqui mostramos que a estratificação nua, seguida de incubação a 30 ° C, pode ter um efeito benéfico significativo na porcentagem de germinação, comprimento da parte aérea e da raiz, bem como na diminuição da incidência de infecções microbianas em nozes-pecã. O efeito positivo da incubação na germinação da noz-pecã observada nesta pesquisa está de acordo com os resultados de trabalhos anteriores. Observou-se que a porcentagem de germinação das castanhas da cv. Wichita foi significativamente superior ao aplicar estratificação nua em comparação com o obtido ao usar estratificação úmida. Os resultados sugerem que muitas sementes de noz-pecã podem dispensar a incubação para completar o processo de germinação, entretanto, a porcentagem de castanhas da cv. Wichita que germinou foi seis vezes maior ao incluir uma fase de incubação no tratamento de pré-germinação. No caso das nozes da cv. Western Schley, a porcentagem de germinação foi 28 vezes maior nas castanhas incubadas do que nas não incubadas. Por outro lado, o presente estudo não conseguiu comprovar a ausência de estratificação para induzir a germinação de nozes pecan, uma vez que todas as nozes utilizadas neste experimento foram armazenadas em refrigeração antes de serem submetidas aos diferentes tratamentos.

**Palavras-chave:** *Carya illinoensis*, semente, Western Schley, Wichita.

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## Introduction

Pecan trees are industrially produced by grafting (Dalkiliç, 2013). It all starts with cross-pollination (Sanderlin, 2015), in which pollen grains from one walnut tree come into contact with pistils from another walnut tree (Ku-Mahamud, 2015; Zheng, Lin, Liang, Wang, & Chen, 2018). ). Once the fertilization of the female gametophyte by the male, it is a matter of time before the pecan nut begins to develop (An, Althiab Almasaud, Bouzayen, Zuine and Chervin, 2020). Then, the mature nuts are collected by the producers, who provide these seeds with the right conditions for them to germinate and, thereby, give rise to new small trees or seedlings (Verma and Chandel, 2017). Subsequently, the trunk of these seedlings is cut to be used as a pattern, so that only the base of the trunk and its roots are preserved. Just after making said cut, a small stem is grafted with bud from a pecan tree in productive age (Casales, Van der Watt and Coetzer, 2018). However, the generation of pecan rootstocks is often complicated by lack of seed uniformity and low germination percentages (Jover, Matta and Shah, 2006). For this reason, strategies should be devised to improve pecan sprouting.

Orchards dedicated to rootstock production should select cultivars that produce large seeds that germinate giving rise to vigorous seedlings capable of favoring graft development in early stages (Sanderlin, 2015). Likewise, it is necessary to determine a method to accelerate the germination process (Zhang, Peng and Li, 2015).

The seeds are classified as orthodox and recalcitrant, according to their tolerance to desiccation. Orthodox seeds are much more tolerant than recalcitrant seeds and can be stored, maintaining their viability for years, while recalcitrant seeds are generally poorly tolerant and die during drying. (Méndez, Covarrubias y Beltrán, 2013).

The seeds of the *Carya* genus are recalcitrant (Dalkiliç, 2013; Poletto, Stefenon, Poletto and Muniz, 2018; Xiao et al., 2020), so that the mature nuts do not go through a dormancy period prior to germination. In fact, by prolonging the storage period, the seeds of the *Carya* genus present a loss of their germination capacity and a decrease in their germination rate (Xiao et al., 2020).

Dalkiliç (2013) worked with pecan nuts cv. Mahan, and found that storing walnuts in a dry environment can slow or inhibit their germination. Even a week in a desiccating environment can be enough for the seeds to lose their ability to germinate.

On the other hand, the seeds of pecan trees can be treated with hormones to promote their germination. For example, in China, a protocol for the propagation of pecan nuts from

seeds has been developed, which achieves that more than 97% of the nuts germinate. First, the seeds are disinfected by immersion in a 3% potassium permanganate solution for 24 hours, then they are soaked in tap water for five days. Subsequently, the seeds are stratified in moist sand, starting in mid-December, for 60 days. Next, the seeds are submerged in a somatropin solution, better known as human growth hormone (Thornton et al., 2021), mixed with 500 mg/L of gibberellic acid, 50 mg/L of indole butyric acid and 50 mg/L of 6-benzyladenine, for 24 hours. Then, the nuts are transferred to the greenhouse to start germination (Zhang et al., 2015).

Another factor with a great impact on the percentage of seed germination is temperature. The biochemical reactions that occur during germination are altered by the presence or absence of light and by the temperature in the environment surrounding the seed (Espindola, Romero, Ruiz and Luna, 2018). For this reason, the term optimum temperature for germination was created, which refers to the temperature at which the highest percentage of germination is achieved in the shortest possible time. This temperature varies according to the species, the cultivar, the genotype and the age of the seed, and can even be altered if the seeds have been exposed to light of different wavelengths, or the application of phytohormones, such as ethylene, acid abscisic and gibberellic acid (Hills y van Staden, 2003).

To the best of our knowledge, no study has been published comparing the effect of bare stratification versus the effect of wet stratification on pecan nuts. For this reason, this research aims to answer the following question: which type of stratification (bare or wet) induces more germination in pecan tree seeds? The hypothesis is that moist stratification will provide higher germination percentages.

## Materials and methods

### Raw material

In October 2019, pecan nuts of the Wichita and Western Schley cultivars (figures 1 and 2) were collected in a 50 ha orchard located in block 1010 of the Yaqui Valley, with geographic coordinates 27° 20' 10.8564" N (latitude ) and 109° 55' 23.04048" W (longitude) (Cd. Obregón, Sonora, Mexico). The nuts were harvested by vibrating the trunk of the pecan tree. Immediately after the tree was shaken, the nuts were collected from the ground. Subsequently, the removal of the husk or husk was carried out, that is, the pericarp, which





appears as a fleshy and green layer that covers the nut, and that dries up, darkens and opens when the almond has matured. The walnuts were then washed and then dried in hot air (30-32°C) for a maximum of four days until the walnut reached a moisture content of 10-12%. Then, the nuts were stored until they acquired an approximate humidity of 3%.

**Figure 1.** Pecan nuts cv. Western Schley



Source: self made

**Figure 2.** Wichita Pecan Nuts



Source: self made

### **Bare stratification, without irrigation, with incubation (ED-SR-CI)**

The nuts were placed in Styrofoam cups and refrigerated at 4 °C for one month. After the stratification period, the nuts were scarified and incubated, according to the methods described below.

### **Stratification in substrate, with irrigation, with incubation (ES-CR-CI)**

The nuts were placed horizontally in Styrofoam cups filled with expanded perlite and refrigerated at 4 °C for one month. The substrate was kept moist by daily irrigation with a 0.2 % fungicide solution, alternating Captan Ultra 50 WP, Benoma-T 50 WP (benomyl) and Velbistin 50 PH (carbendazim). After the stratification period, the nuts were scarified, and finally they were incubated.

### **Bare layering, no irrigation, no incubation (ED-SR-SI)**

This stratification constituted the control treatment. The nuts were placed in Styrofoam cups and refrigerated at 4 °C for one month. Subsequently, the nuts were scarified and planted individually in bags filled with a mixture of peat moss, vermiculite and perlite (5:3:2; V:V:V), and were kept for 17 days in a refrigerated greenhouse. The substrate was kept moist by irrigation with tap water.

## **Scarification**

To soften the shell and detach it from the kernel, the nuts were soaked in a fungicidal solution of Captan Ultra 50 WP and Velbistin 50 PH (carbendazim) (1:1, 2 g/L) for 10 hours, under a constant supply of air, using a fish tank aerator. Then, using a nutcracker, the shell was removed from half of the nut, exposing the end of the embryonic axis, where the root and the seed shoot emerge, as can be seen in figure 3.



**Figure 3.** Pecan partially scarified



Source: self made

### **Incubation**

The nuts were kept at 30-32 °C for 17 days. During incubation, the substrate was kept moist by applying a 0.2% fungicide solution, alternating Captan Ultra 50 WP, Benomyl 50 WP (benomilo) y Velbistin 50 PH (carbendazim).

### **Evaluation of walnut germination**

At the end of each treatment, the percentage of germinated nuts and the percentage of contaminated nuts were calculated. Likewise, the length of the roots and shoots of the germinated nuts was measured using a tape measure.

### **Evaluation of microbial contamination in walnuts**

The evaluation of the contamination of walnuts by microorganisms was carried out by visual examination of the same. Contaminated walnuts were counted and the percentage of contaminated walnuts was calculated considering the 55 walnuts of each replica as the total number of walnuts.

## Statistic analysis

The treatments were distributed in a completely randomized design. Three replicates of each treatment were made, and a sample size of 55 nuts ( $n = 55$ ) was used for each replicate. Data were subjected to a one-way analysis of variance (ANOVA), considering the type of pre-germination treatment (type of stratification in combination with incubation or no incubation) as the independent variable. Differences between means were determined by Duncan's test ( $p \leq 0.05$ ). The data was analyzed with the statistical package IBM SPSS Statistics, version 22.

## Results

The average weight of the nuts of cvs. Wichita and Western Schley prior to the application of pregerminative treatments was  $9.3576 \text{ g} \pm 1.0581 \text{ g}$  and  $8.7010 \text{ g} \pm 1.2753 \text{ g}$ , respectively. The germination results of the walnuts appear in table 1.

**Table 1.** Percentage of germination and contamination, as well as root and shoot length in pecan nuts of Wichita and Western Schley cultivars, depending on the pre-germination treatment

Tratamiento	Cultivar	Germinación (%)	Contaminación (%)	Longitud de raíz (cm)	Longitud de brote (cm)
ED-SR-CI	Wichita	$69.69 \pm 2.77^a$	$18.78 \pm 10.01^a$	$1.84 \pm 0.03^a$	$1.83 \pm 0.01^a$
	Western Schley	$51.51 \pm 4.57^b$	$59.39 \pm 5.55^b$	$1.08 \pm 0.19^{b,c}$	$1.14 \pm 0.13^{b,c}$
ES-CR-CI	Wichita	$56.35 \pm 4.80^b$	$48.48 \pm 5.55^b$	$1.16 \pm 0.08^b$	$1.26 \pm 0.09^b$
	Western Schley	$51.51 \pm 8.20^b$	$55.75 \pm 6.88^b$	$0.98 \pm 0.09^{b,c}$	$0.96 \pm 0.14^{c,d}$
ED-SR-SI	Wichita	$10.90 \pm 1.81^c$	$100 \pm 0.00^c$	$0.94 \pm 0.09^c$	$0.76 \pm 0.13^d$
	Western Schley	$1.81 \pm 1.81^d$	$100 \pm 0.00^c$	$0.08 \pm 0.07^d$	$0.06 \pm 0.05^e$

\*ED-SR-CI: bare stratification without irrigation, with incubation; ES-CR-CI: stratification in perlite, with irrigation, with incubation; ED-SR-SI: bare stratification without irrigation, without incubation, with greenhouse sowing. Values are the mean  $\pm$  standard deviation of percentage of germinated nuts, percentage of contaminated nuts, root length, and shoot

length of three replicates of 55 nuts each ( $n = 55$ ). Data found in the same column and sharing the same superscript show statistically insignificant differences according to Duncan's test (Anova,  $p < 0.05$ )

Source: self made

### **Germination differences depending on the cultivar of origin**

In the present study, the percentage of germination in the walnuts of the Wichita cultivar was significantly higher than that of the walnuts of the Western Schley cultivar in the treatments of bare stratification without irrigation, with incubation (ED-SR-CI) and bare stratification without irrigation, without incubation, with greenhouse sowing (ED-SR-SI). There were no significant differences between the germination percentage of the nuts of both cultivars that received the perlite stratification treatment with irrigation, with incubation (ES-CR-CI). Likewise, the average root length in the germinated walnuts of the Wichita cultivar was significantly higher than that observed in the walnuts of the Western Schley cultivar in the ED-SR-CI and ED-SR-SI treatments. In addition, the mean shoot length in germinated walnuts of the Wichita cultivar was significantly higher than that observed in walnuts of the Western Schley cultivar in all three treatments tested.

### **Microbial contamination according to cultivar**

In the present investigation, with the ED-SR-CI treatment, the walnuts of the Wichita cultivar presented a significantly lower incidence of fungal contamination than the walnuts of the Western Schley cultivar. Additionally, the highest percentage of microbial contamination was observed in the seeds to which no fungicide was applied and which were sown in a greenhouse, which proves the effectiveness of the combination of fungicides applied during incubation.

### **Effect of temperature on germination**

The walnuts of both cultivars that were incubated (ED-SR-CI and ES-CR-CI treatments) showed a significant increase in the germination percentage, as well as in the shoot and root length, compared to the walnuts that only they were stratified and placed in substrate to germinate in the greenhouse (ED-SR-SI). This may be because the incubating nuts were in a constant warm temperature environment. However, the incidence of microbial

contamination is probably the single most detrimental factor in nut germination and development.

Considering the results presented in table 1, incubation allowed a higher percentage of seeds to germinate. In the case of walnuts of cv. Wichita, up to an additional 58.79% of walnuts managed to germinate when an incubation stage was included in the pre-germination process, and in the case of walnuts of cv. Western Schley up to an additional 49.7% of nuts germinated due to incubation.

The impact of temperature during germination could have been verified with the addition of a greenhouse germination treatment that included an initial period of 17 days of irrigation with fungicide, as was done during the incubation of the nuts of the other treatments.

### **Effect of the type of stratification on germination**

Taking into account the results of table 1, it was observed that the percentage of germination in the nuts of cv. Western Schley was not affected according to the type of stratification applied, however, it was found that an additional 13.34 % of nuts of cv. Wichita germinated by bare stratification, compared to walnuts that received wet stratification.

## **Discussion**

### **Germination differences depending on the cultivar of origin**

It is evident that the results of germination depend to a great extent on the cultivar of the parent plant. As was shown in the study by Rehman, Hussain, Zaib-un, and Awan (2000); they grew pecans from the cvs. Wichita, Mohan, Shawnee, Burket and Mohawk without applying pregerminative treatments. The cv. Burket had the highest averages for root and shoot length, as well as number of roots. The cv. Mohan had the lowest averages of these variables. Regarding germination, the highest percentage was observed in the nuts of cv. Burket (82%), followed by cv. Wichita (80%). On the other hand, it was found that the time required for seed germination varied from one cultivar to another; the seeds of cv. Wichita were the slowest, requiring 114 days to complete germination, while nuts of cv. Mohan were the fastest, with 101 days.

On the other hand, the stratification process can give different results depending on the walnut cultivar, as was observed in the study by Jover et al. (2006). In this investigation, six pre-germination treatments were tested on pecan cvs. Hughes and Owens, which consisted of three different stratification times at 3 °C and three different storage times at 10 °C; Additionally, some nuts were planted just after harvest. The cultivars studied responded in the opposite way to stratification: with the increase of the stratification period, the walnuts of the cultivar Owens suffered a decrease in the percentage of germination, while a higher percentage of walnuts of the Hughes cultivar germinated when the stratification was prolonged. On the other hand, the nuts of both cultivars suffered a loss of their germination capacity as the storage time at 10 °C was prolonged. Furthermore, it was observed that the stratified walnuts had a significantly higher germination percentage than the stored walnuts.

### **Microbial contamination according to cultivar**

It is possible to identify cultivars of a certain plant species that are more resistant to fungal infections (Kabak, Dobson and Var, 2006). For example, the level of fungal contamination in bean (*Phaseolus vulgaris*) seedlings generated from the seeds of 11 different cultivars has been analyzed, and it has been found that colonization by endophytic fungi differs significantly between cultivars (Parsa et al., 2016 ).

In the specific case of *C. illinoensis*, the nuts of the Western Schley cultivar have a low germination percentage, because they tend to rot in the stratification stage, and their use for the generation of rootstocks is discouraged (Conner, 2010).

Apart from Western Schley, other pecan cultivars have been identified whose offspring are susceptible to microbial contamination. Sanderlin (2015) worked with patterns from seeds obtained by cross-pollination from eight pecan cultivars, namely: Apache, Curtis, Elliott, Moore, Riverside, Stuart, VC1-68 and Cape Fear. Seedlings were inoculated with *Xylella fastidiosa* to determine their tolerance to infection by this pathogenic bacterium. The eight cultivars studied were susceptible to infection by *X. fastidiosa*, but it was found that in the case of cv. Apache a significantly higher percentage of seedlings showed signs of infection, while in the case of cv. Curtis, a significantly lower percentage of infected seedlings was observed, compared to the results obtained in the rest of the cultivars.

## Effect of temperature on germination

Seeds of the *Carya* genus have higher germination percentages and more uniform germination rates when incubated at 30-35 °C (Xiao et al., 2020). The walnut shell is permeable to water and gases, but it retards germination by imposing a mechanical constraint on radicle elongation. This restriction can be overcome by incubating the seed at optimum temperature (30-35°C). At these temperatures, germination is uniform and rapid, and is completed in less than 20 days (van Staden and Dimalla, 1976).

Van Staden and Dimalla (1976) investigated the effect of incubation at different temperatures (20, 25, 30 and 35 °C) on germination of scarified and intact pecans, cvs. Curtis, Major and Peruque. Scarification, by itself, did not result in an increase in the final percentage of germination. However, scarifying and incubating the nuts at 30-35°C made germination uniform and germination percentages high. Scarified walnuts reached maximum germination in 12 days, compared to intact walnuts, which reached maximum germination in 22 days.

The combined effect of stratification, imbibition, and warm incubation on pecan germination has also been tested. Bustamante, Gonzalez, Benavides, Banuelos and Rojas (2006) applied pre-germination treatments to pecans cv. Western Schley and then planted them in substrate and distributed them in a greenhouse. They found that walnuts soaked in water and incubated at 30 °C germinate faster and develop taller seedlings in less time, compared to walnuts that have only been soaked or walnuts that have been soaked and stratified. However, it was found that, after some time, the seedlings obtained by incubation stopped growing, while the other seedlings continued to grow and exceeded the height of the seedlings generated by incubation. It is speculated that the application of heat during incubation inhibited seedling growth because it induced the synthesis of abscisic acid and jasmonic acid.

In another investigation, it was observed that pecans that were not stratified and that were incubated at 20 °C had a low percentage of germination. In contrast, 95% of the nuts that were incubated at 30 °C germinated in just 22 days. Similarly, the walnuts that were stratified at 4 °C for 90 days and subsequently incubated at 20 °C had a high percentage of germination in a short time. With these results, it was concluded that pecans require stratification or incubation at 30 °C to germinate properly (Dimalla and van Staden, 1977). These conjectures agree with the results of the present investigation, where the highest percentage of germination, the lowest percentage of contamination and the longest root and



shoot lengths were observed in the walnuts of the Wichita cultivar that received the ED-SR-CI treatment.

In the past, there was a notion that pecan germination in the wild and in nursery soil is sporadic because these environments do not reach high enough temperatures. It was considered that the heat requirement for the induction of walnut germination could be due to the fact that the lipid reserves of the seed are better mobilized at high temperatures (van Staden and Dimalla, 1976).

It is now known that seed storage tissues contain nutrients that must be hydrolyzed in order to be transported to the sites where they are required as an energy source during germination (Bewley, Bradford, Hilhorst and Nonogaki, 2013). Particularly, in walnuts the reserve organs are the cotyledons (Scussel, Manfio, Savi and Moecke, 2014) and their main reserve substance are lipids (Flores et al., 2017). Therefore, the activity of lipases is essential for the germination of nuts, since lipases are glycerol ester hydrolases that catalyze the hydrolysis of triacylglycerols to free fatty acids and glycerol. (Joseph, Ramteke y Thomas, 2008).

There are studies where the activity of lipases in pecans of the Wichita and Western Schley cultivars has been quantified (Tanwar, Modgil and Goyal, 2020; Wakeling, Mason, D'Arcy and Caffin, 2001). These results could be used to categorize the seeds of different cultivars or those subjected to different pre-germination treatments, establishing a relationship between the activity of lipases and the length of the shoot generated in germination, since there is evidence that the mobilization of reserves of the seed may be associated with the vigor of the seedling (Walters, Landré, Hill, Corbineau and Bailly, 2005).

One of the factors that most alter the activity of lipases is temperature. Barros, Fleuri and MacEdo (2010) identified the optimal temperature for the activity of commercial lipases extracted from seeds of 14 plant species. In this study, all the lipases examined presented an optimal activation temperature equal to or greater than 30 °C.

Once the activity of the lipases is ensured, a series of metabolic events is triggered. First, triacylglycerols are hydrolyzed to diacylglycerols, then monoacylglycerols, and finally to free fatty acids and glycerol, by the action of lipases; then, glycerol is converted to dihydroxyacetone phosphate by the action of the enzymes glycerol kinase and glycerol-3-phosphate dehydrogenase; subsequently, the free fatty acids are transported to the glyoxysome where they acquire the active form of acyl coenzyme A; then, acyl coenzyme A

goes through a  $\beta$ -oxidation process, to produce acetyl coenzyme A; Acetyl coenzyme A then enters the glyoxylate cycle, and is converted to organic acids; later, the organic acids pass to the mitochondria and the cytosol, where they are converted into oxaloacetic acid; Finally, oxaloacetic acid and dihydroxyacetone phosphate go through a process of gluconeogenesis to produce sucrose, which is mainly transported to the embryonic axis. (Bewley *et al.*, 2013).

## Conclusions

Evidence is presented that bare stratification provides significantly superior results to wet stratification. Specifically, bare stratification, followed by incubation at 30-32 °C, had a significant beneficial effect on germination percentage, shoot and root length, as well as decreased incidence of microbial infections in pecan nuts. Likewise, the evidence indicates that cv. Wichita outperforms cv. Western as a seed source for rootstock production.

The results imply that many pecan seeds can do without incubation to complete the germination process, however, the percentage of nuts of cv. Wichita that germinated was six times higher when including an incubation stage in the pre-germination treatment. In the case of walnuts of cv. Western Schley, the percentage of germination was 28 times higher in the incubated nuts than in the nuts that were not incubated. On the other hand, the present study was unable to prove the absence of stratification to induce germination of pecan nuts, since all the nuts used in this experiment were stored in refrigeration before being subjected to the different treatments.

## Future lines of research

For future research, it is proposed to test the application of prochloraz fungicide solution for seed hydration during incubation. In addition, in order to verify that the benefits of incubation are due to the maintenance of a warm temperature and not to the constant application of fungicide, it is proposed to test the effect of sowing the seeds in a greenhouse without previously stratifying or incubating them, but including irrigation. with fungicide. Finally, it is recommended that in the next germination experiments the survival and vigor of the seedlings be evaluated at one year of age in order to know the effectiveness of the treatments in the medium term.

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