Notas Científicas

Germination and development of pecan cultivar seedlings by seed stratification

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Abstract – The objective of this work was to evaluate the effect of seed stratification on germination rate, germination speed, and initial development of seedlings of six pecan (*Carya illinoinensis*) cultivars under subtropical climatic conditions in southern Brazil. For stratification, the seeds were placed in boxes with moist sand, in a cold chamber at 4°C, for 90 days. In the fourteenth week after sowing, the emergence speed index, total emergence, plant height, stem diameter, and number of leaves were evaluated. Seed stratification significantly improves the germination potential and morphological traits of the evaluated cultivars.

Index terms: *Carya illinoinensis*, dormancy break, germination, rootstock, seedling quality.

Germinação e desenvolvimento de mudas de cultivares de nogueira-pecã com estratificação de sementes

Resumo – O objetivo deste trabalho foi avaliar o efeito da estratificação de sementes na taxa de germinação, na velocidade de germinação e no desenvolvimento inicial de mudas de seis cultivares de nogueira-pecã (*Carya illinoinensis*) sob condições climáticas subtropicais, no Sul do Brasil. Para estratificação, as sementes foram acomodadas em caixas com areia úmida, em câmara fria a 4°C, por 90 dias. Na décima quarta semana após o plantio, foram avaliados o índice de velocidade de emergência, a emergência total, a altura da planta, o diâmetro do caule e o número de folhas. A estratificação das sementes melhora significativamente o potencial de germinação e as características morfológicas das cultivares avaliadas.

Termos para indexação: *Carya illinoinensis*, quebra de dormência, germinação, porta-enxerto, qualidade de mudas.

The pecan [*Carya illinoinensis* (Wangenh) K. Koch], from the Juglandaceae family, is a deciduous tree species native to the temperate zones of North America, introduced with commercial interest in Brazil during the 1870s. However, cultivating exotic species demands the selection of cultivars and rootstocks adapted to the climatic and biological conditions where the orchards are established. The major limiting factor for nursery production of pecan is the restricted availability of rootstocks. Typically, selected cultivars are grafted over rootstocks produced by means of seeds collected from open-pollinated pecan cultivars, and are usually limited by low and irregular germination.

Low and irregular germination may be caused by seed dormancy. In addition, the time for seedling production is extended (about 3 years are required to obtain vigorous seedlings from the pecan seeds) and the predisposition to phytosanitary problems is increased. The existence of seed dormancy in pecan is still a controversial topic. Adams & Thielges (1978) and Ghazaeian et al. (2012) proposed to overcome the dormancy of pecan seeds by maintaining seeds at 3 to 7°C. However, Smith et al. (1997) reported increased germination rate with stratification at 32°C. In Brazil, seedlings are usually produced without any kind of seed stratification.

Although pecan was introduced in the country more than a century ago, few studies on seed technology of this species have been performed, considering the local environment, and only some have proposed the presence of embryo dormancy in the species (Dimalla & Van Staden, 1978).

The objective of this work was to evaluate the effect of seed stratification on germination rate, germination speed, and initial development of seedlings of six pecan (*Carya illinoinensis*) cultivars under subtropical climatic conditions in southern Brazil.

The study was performed in a 50 m^2 nursery environment, in the municipality of Anta Gorda, in the state of Rio Grande do Sul, Brazil (28°53'S, 52°02'W, at 537 m above sea level). The climate of the region is Cfa, according to the Köppen, subtropical moist, without dry season and with a mean annual precipitation of 1,800 mm. According to the Brazilian soil classification (Santos et al., 2013), the soil is a Chernossolo Argilúvico (Typic Argiudoll). The experiment was conducted using a completely randomized design with a factorial arrangement consisting of stratified and non-stratified seeds and of six cultivars (Barton, Barton2, Desirable, Importada, Maham, and Melhorada), with five replicates for each cultivar. All seeds were collected from five trees of each cultivar in the orchards established in the municipality of Anta Gorda. Seeds were dried during 3 weeks in a shadowed environment; however, only the ones with mass over 8.0 g were used in the experiment.

The stratification treatment consisted in maintaining seeds in 5.0-cm layers of seeds, alternated with 5.0-cm layers of sand:sawdust (1:1; volume basis), within wood boxes. The boxes were placed in a cold chamber at 4±0.1ºC. For the control treatment (without stratification), seeds were maintained at room temperature in paper bags. After 90 days, sowing was performed manually by placing each seed in a 3.0-cm-deep spot, with a distance of 50 cm between rows and 20 cm between spots. Irrigation was performed through a dripping system, and weeds were manually controlled. Fourteen weeks after sowing, plantlet emergence (%), plant height (cm), stem diameter (mm), and the ratio between plant height and diameter (H/D) were recorded for all plants. Plant emergence was checked weekly, and the emergence speed index was estimated according to Maguire (1962).

The analysis of variance was performed to compare the effect of treatments on the evaluated parameters. Data on plantlet emergence and the emergence speed index were transformed prior to statistical analysis, by $(x+0.5)^{0.5}$. When the analysis of variance was significant, Tukey's test, at 5% probability, was used to compare differences between the means of treatments, using the software Sisvar, version 5.3 (Ufla, Lavras, MG, Brazil).

A significant effect was observed for each isolated factor, except for the H/D ratio for the cultivars ($p=0.19$). There was no interaction between cultivar and treatments ($p>0.16$), and also no difference among cultivars regarding percentage of emergence, stem diameter, number of leaves, and H/D ratio for the stratified seeds. For the emergence speed index, the Mahan cultivar presented significantly lower values in comparison to Barton, Melhorada, and Barton2. Regarding plant height, the Mahan cultivar had significantly lower values than Barton and Barton2 (Table 1). The absence of the interaction cultivar-treatment and the lack of differences among cultivars for almost all traits suggest that the stratification treatment may also be efficient for other cultivars not included in the present study.

In comparison to the control treatment, the stratified seeds presented superior results for all evaluated traits, except for the H/D ratio for the Barton, Importada, Melhorada, and Barton2 cultivars (Table 1). Stratified seeds presented values from 70 to 100% for emergence, in comparison to the control treatment, with a mean value of 32.8%. For the emergence speed index, stratified seeds showed a mean value of 16, whereas the control, a mean value of 1.7. Plantlet emergence in the stratified seeds started between the second and third weeks after sowing, with emergence peak between the third and sixth weeks, stabilizing in the seventh week (Figure 1). In the control treatment, emergence started between the fifth and seventh weeks, and it was not possible to distinguish the emergence peak nor the stabilization period. In the fourteenth week, plantlet emergence ranged from 70 to 100% for the stratified seeds and from 15 to 50% for the control treatment. These results may suggest the need of cold stratification for enhancing germination in pecan cultivars planted in southern Brazil. The need of low temperatures (3 to 7°C) to overcome dormancy in pecan is expected and recommended for orchard establishment in Iran (Ghazaeian et al., 2012) and even in the USA (Adams & Thielges, 1978). Cold stratification at 4°C also

Table 1. Emergence, emergence speed index (ESI), height, diameter, number of leaves, and height/diameter (H/D) ratio of stratified and non-stratified (control) seedlings of different pecan (*Carya illinoinensis*) cultivars[(1)].

Cultivar	Emergence (%)		ESI		Height (cm)		Diameter (mm)		Number of leaves		H/D ratio (cm mm^{-1})	
	Stratified	Control	Stratified	Control	Stratified	Control	Stratified	Control	Stratified	Control	Stratified	Control
Barton	96.7aA (8.4)	46.7aB (22.1)	18.0aA (14.3)	2.1aB (51.3)	29.6aA (14.7)	18.1abB (25.1)	8.7aA (11.7)	5.4abB (19.8)	17.3aA (10.8)	12.4aB (20.3)	3.4aA (8.8)	3.4aA (16.1)
Melhorada	93.3aA (11.1)	33.3abB (35.4)	18.5aA (15.4)	1.9aB (88.0)	27.3abA (14.4)	15.7abB (27.0)	9.8aA (10.0)	4.9abB (37.4)	17.2aA (7.6)	10.4abB (37.3)	2.8aA (7.0)	2.4aA (27.0)
Importada	96.7aA (8.4)	33.3abB (23.1)	15.4abA (18.2)	1.3aB (94.1)	26.5abA (12.4)	10.3bB (48.4)	7.9aA (13.3)	3.1bB (44.9)	14.0aA (9.7)	7.3abB (28.7)	3.3aA (5.4)	2.3aA (7.5)
Mahan	73.3aA (22.3)	16.7bB (40.0)	12.0bA (30.8)	0.9aB (118.3)	23.0bA (15.7)	9.8bB (43.1)	7.3aA (21.8)	3.4abB (37.1)	13.0aA (13.1)	8.8abB (32.2)	3.6aA (12.7)	1.9aB (21.0)
Barton 2	86.7aA (18.8)	50.0aB (42.0)	16.9aA (12.1)	3.4aB (64.1)	33.7aA (11.0)	21.6aB (13.6)	10.2aA (5.9)	6.3aB (14.0)	16.6aA (8.2)	12.4aB (17.0)	3.3aA (8.2)	3.4aA (8.6)
Desirable	80.0aA (15.8)	16.7bB (69.3)	15.2abA (25.2)	0.6aB (112.9)	29.7abA (11.5)	8.2bB (19.1)	9.3aA (7.9)	2.3bB (12.9)	14.3aA (6.8)	4.9bB (7.1)	3.2aA (11.7)	1.7aB (19.0)
Mean	87.2	32.8	16.0	1.7	28.3	15.2	8.9	4.3	15.4	9.4	3.3	2.5
CV (%)	22.5		18.3		26.1		21.3		21.9		30.3	

[(1)]Means followed by equal letters, uppercase in the rows and lowercase in the columns, do not differ by Tukey's test, at 5% probability. Coefficient of variation (CV) of the cultivar for each trait between parenthesis.

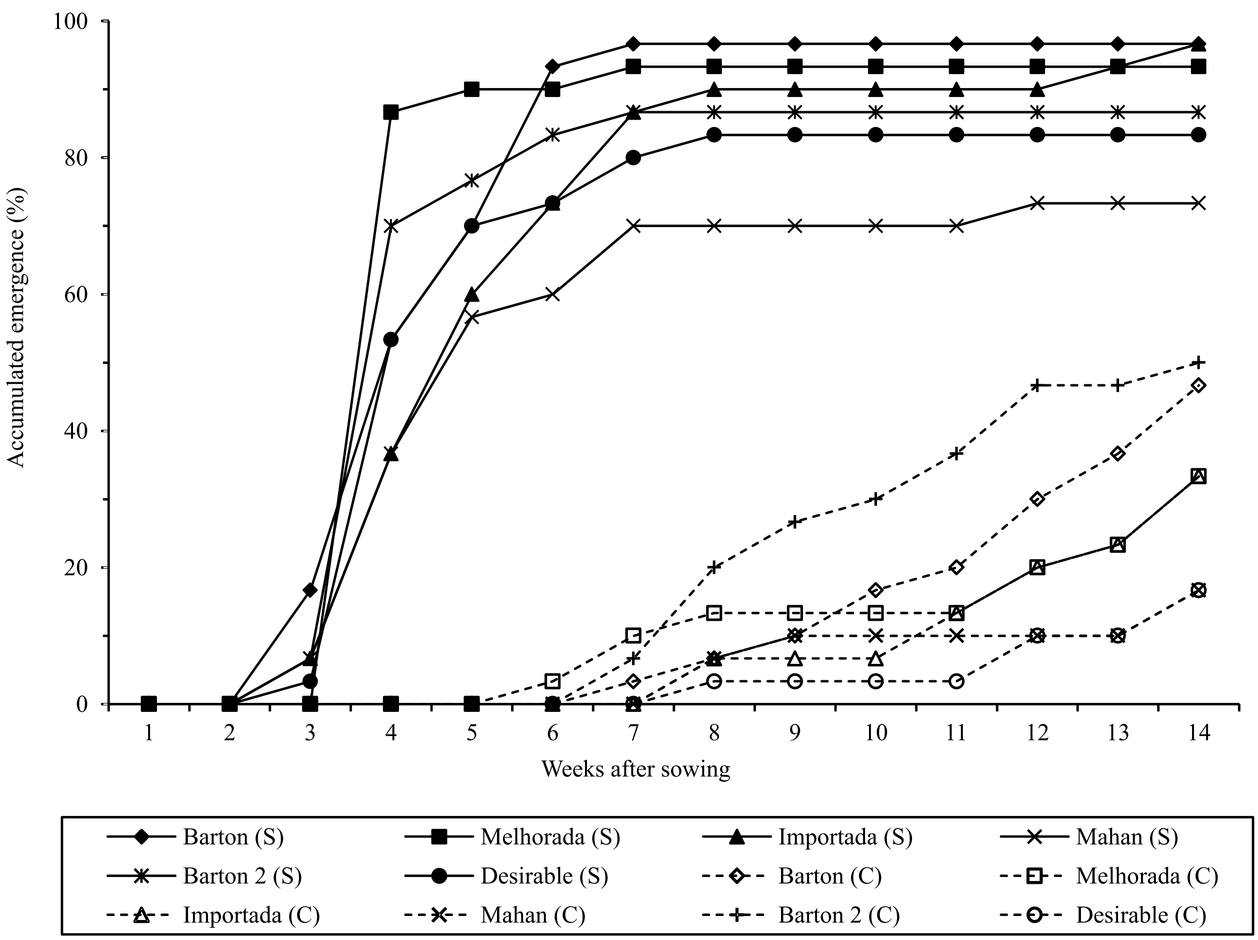

Figure 1. Accumulated emergence of stratified (S) and non-stratified (C) seeds from different pecan (*Carya illinoinensis*) cultivars. Non-stratified seeds represent the control treatment.

increased germination of *Lupinus sulphureus* in the USA and of *Cercis siliquastrum* in Greece to rates higher than 70% (Kaye & Kuykendall, 2001; Pipinis et al., 2011).

The mean plant height observed in the stratification treatment was 28.3 cm, whereas the control treatment generated plants with a mean height of 15.2 cm. For stem diameter, the observed mean was 8.9 cm for the stratification treatment and 4.3 cm for the control. For all cultivars, the mean number of leaves (Table 1) was larger for the stratification treatment (15.5 leaves per plant), when compared to the control treatment (12.2 leaves per plant). A significant difference between stratification and control treatments was found for the Mahan and Desirable cultivars for the H/D ratio. For the other cultivars, the H/D ratio was higher than 2.3 cm mm^{-1}, and no significant difference between the stratification and the control treatments was observed. For all traits, the coefficient of variation showed homogeneity of the plantlets subjected to the stratification treatment, and a high coefficient of variation was observed for all traits in the control treatment (Table 1).

Overall, the stratification treatment using low temperature was crucial for the homogeneity and superior behavior of the plantlets for all evaluated cultivars, in comparison to the control treatment. Effective seed stratification using low temperatures was also reported for *Cupressus lusitanica* (Xavier et al., 2012) and *Chaenomeles sinensis* (Entelman et al., 2009), aiming at cultivation in the subtropical climatic conditions of southern Brazil.

The use of cold stratification of seeds is needed to overcome the dormancy of pecan, with significantly superior results obtained for all cultivars. At nurseries, this will result in higher quality seedlings and in greater homogeneity, enabling grafting at the same time for a larger amount of plantlets, reducing costs for seedling commercialization.

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