

*Translated from the original in spanish*

## Morphological structure, germination and vigor of *Juglans jamaicensis* c. dc. seeds in the Turquino National Park

### Estructura morfológica, germinación y vigor de semillas de *Juglans jamaicensis* C. DC. del parque nacional turquino

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#### ABSTRACT

Seven groups of *Juglans jamaicensis* trees were selected in Parque Nacional Turquino to determine the morphological structure, germination power and vigor of *Juglans jamaicensis* seeds. To do this, the seeds were collected from the soil and subjected to pregerminative treatment, then 900 of them were placed in the germinator under a completely randomized design. The morphology of the seeds was evaluated from the parameters length of the major axis, diameter, weight and volume of the seed. The germination control started from the seventh day to 90 days. Germination capacity, germination dynamics, and seed vigor were determined through germination speed and Czabator indices. The morphological variation of seeds in relation to progenitor trees and sites, as well as the effect of parental origin on germination, germination dynamics and seed vigor was checked by means of a simple classification ANOVA, and the HSD-Tukey test. The structure of *Juglans jamaicensis* seeds is an adaptation of trees to the environment in which they develop in response to environmental variation and the establishment of *Juglans jamaicensis* regenerants in natural conditions, it is determined by high germination potential ( $73\pm16\%$ ), low germination speed ( $0.40\pm0.1$ ) and vigor ( $0.38\pm0.3$ ) as well as offspring anomalies, which are key elements leading to a potential decrease in the abundance of seedlings in the forest.

**Keywords:** walnut cedar; *Juglans jamaicensis*; germination capacity.

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#### RESUMEN

Se seleccionaron siete grupos de árboles de *Juglans jamaicensis* en el Parque Nacional Turquino para determinar la estructura morfológica, poder germinativo y vigor de las semillas. Para ello se recolectaron las semillas del suelo y fueron sometidas a tratamiento pregerminativo, posteriormente 900 de ellas se colocaron en el germinador bajo un diseño completamente aleatorizado. Se evaluó la morfología de las semillas a partir de los parámetros longitud del eje mayor, diámetro, peso y volumen de la semilla. El control de la germinación comenzó a partir del séptimo día hasta los 90 días. Se determinaron la capacidad germinativa, la dinámica de la germinación, y el vigor de las semillas a través de los índices de velocidad de germinación y de Czabator. La variación morfológica de las semillas en

relación a los árboles progenitores y los sitios, así como el efecto del origen parental sobre la germinación, la dinámica de germinación y el vigor de las semillas se comprobó mediante un ANOVA de clasificación simple, y la prueba HSD -Tukey. Como resultados se obtuvo que la estructura de las semillas constituye una adaptación de los árboles al medio en que se desarrollan como respuesta a la variación ambiental, además que el establecimiento de los regenerantes de *Juglans jamaicensis* en condiciones naturales, está determinado por un alto potencial germinativo ( $73\pm16\%$ ), baja velocidad germinativa ( $0,40\pm0,1$ ) y vigor ( $0,38\pm0,3$ ) así como anomalías en la descendencia, que constituyen elementos clave conducentes a una disminución potencial de la abundancia de plántulas en el bosque.

**Palabras clave:** cedro nogal; *Juglans jamaicensis*; capacidad germinativa.

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## INTRODUCTION

*Juglans jamaicensis* C. DC, is one of the most important species from the timber and ecological point of view of the Antilles, for the quality of its wood and its state of endangered species (González et al., 2016). Since 1923 its cutting is prohibited by the decree Law 979 of July 4, 1923.

It is known that this species, prefers shaded places in the ravines, next to torrents and ravines of the mountainous regions; According to Bibb and Monsegur (2013), it is gregarious and of scarce appearance in the vegetation as a result of the depression of its populations (aspect that has been considered to declare it as threatened), as well as the alteration of its habitat by the cultivation of coffee in the mountains and the irrational extraction in search of the quality of its wood, to which are added the avalanches or landslides in the mountains and the impact of climate change seen through the increase of extreme meteorological events such as hurricanes.

In spite of the fact that the species has been protected in Cuba and in the program of Forest Development until 2015 of the province of Granma, a group of strategies have been outlined to give solution to insufficiencies. On the other hand, *Juglans jamaicensis* has shown a high germination capacity, as well as scarce regeneration in natural conditions, depression of its individuals by the action of lianas and the illegal felling of its major trees.

Therefore, it was identified as an objective to determine the morphological structure, germinative power and vigour of the seeds of *Juglans jamaicensis*, an endangered species of the Cuban mountain flora.

## MATERIALS AND METHODS

Seeds were selected from seven groups of *Juglans jamaicensis* trees in the Unidades Zonales de Conservación Santo Domingo and La Platica, belonging to Parque Nacional Turquino. The seven sites were: in UZC Santo Domingo: Jeringa Arriba (JA), Jeringa Abajo (JB), Armando Osorio (AO) and in UZC La Platica: Minihidroeléctrica (MH), Rolando Arriba (RA), Rolando Abajo (RB) and Altos de Palma Mocha (PM).

## Morphological structure of seeds

In order to characterize the morphological variation of seeds in relation to progenitor trees and sites, soil seeds were collected in August 2013, morphometric parameters of major axis length (seed length (LS)) and seed diameter (DS) were measured with a caliper of 0.02 mm precision, as well as weight in grams of 100 seeds (PS). The seed volume (VS) was also determined by approximating it to the volume of a cylinder according to the formula: Seed volume=long\*\*((diameter/2)<sup>2</sup>)<sup>π</sup> (Arteaga, 2007). This parameter was transformed to Ln for adjustment to normal distribution. The results were subjected to a variance analysis (ANOVA) and the HSD-Tukey test with a significance level of 5 %, using SPSS version 21.0.

## Germination and vigor of the seeds of *Juglans jamaicensis* C. DC

For the germination test the seeds were submitted to the pregerminative treatment recommended by Pérez *et al.*, (2015), which consisted in soaking them in water at room temperature for 24 hours, with water change every 12 hours; later to evaluate the germinative capacity in relation to the progenitor trees, they were placed in the open-air germinator of the Estación Experimental Agro-Forestal de Guisa, on a substrate formed by 70 % of sand and 30 % of pine sawdust, following a completely randomized design, with four repetitions and 25 seeds per repetition for a total of 900 seeds.

The germination capacity was determined with the germination control that started from the seventh day, carrying out a daily count. Observations were made until 90 days. Irrigation was carried out on alternate days, preferably in the afternoon, with the use of a watering can without prior control of environmental variables.

To evaluate the dynamics of germination, the parameters t0 (number of days elapsed between the moment of sowing and the beginning of germination) and t50 (the time elapsed, in weeks, from when seeds are sown until 50 % germination is reached) were determined (Sánchez *et al.*, 2017), as well as the germination speed index (IVG) (Carrillo *et al.*, 2017) and the Czabator index (IC) to determine the vigour of the seeds (López and Gil, 2017). During the germination process the presence of anomalies in the plants were observed both in the roots and in the aerial part.

The effect of the progenitor tree on germination, germination dynamics and seed vigour was determined by means of a simple classification ANOVA, followed by the HSD-Tukey multiple comparison test. In order to adjust the data to a normal distribution, the CG variable was transformed to (CG) using the SPSS program version 21.0 for Windows.

## RESULTS AND DISCUSSION

### Morphological structure of seeds

The quality of forest seed depends on several factors including size and weight. The results show that there are significant differences between these factors and their origin.(Table 1).

With respect to length and diameter it was found that the seeds of Rolando Arriba were the longest and widest, globular in appearance compared to those of Rolando Abajo, Jeringa Abajo, Palma Mocha 3 and Jeringa Arriba, while the seeds of Palma Mocha 2, Armando Osorio and Palma Mocha 1 showed an intermediate size.

Regarding the weight of the seeds, the trees of Rolando Arriba (6.6 g) and Rolando Abajo (6.04 g) produced the heaviest ones, while those of Palma Mocha were of lesser weight (3-4 g). The rest of the trees formed seeds of intermediate weight (5 g).

Comparing the length, diameter and weight of seeds from the TNP with those reported in the literature, it was found that these parameters were higher than those reported by Álvarez *et al.*, (2006) [LS= 1.5 - 1.9 cm; DS= 0.9 - 2.1 cm; PS= 1.3 - 3.9 g] responding perhaps to the variability within the species related to the sites where they are found.

**Table 1.**- Morphological parameters of *Juglans jamaicensis* seed quality

Origen parental	LS (cm; media±DE)	DS (cm; media±DE)	PS (g; media±DE)	VS (cm <sup>3</sup> ; media±DE)
<b>PM 1</b>	2,34±0,15 <sup>b</sup>	1,99±0,11 <sup>e</sup>	3,20±0,59 <sup>f</sup>	7,37±0,79 <sup>f</sup>
<b>PM 2</b>	2,48±0,14 <sup>a</sup>	2,02±0,14 <sup>e</sup>	3,70±0,83 <sup>e</sup>	7,88±0,88 <sup>e</sup>
<b>PM 3</b>	2,19±0,14 <sup>d</sup>	2,43±0,19 <sup>b</sup>	4,22±0,54 <sup>d</sup>	8,42±1,05 <sup>cd</sup>
<b>RA</b>	2,47±0,92 <sup>a</sup>	2,58±0,12 <sup>a</sup>	6,64±1,08 <sup>a</sup>	10,05±0,73 <sup>a</sup>
<b>RB</b>	2,16±0,08 <sup>d</sup>	2,56±0,13 <sup>a</sup>	6,04±1,08 <sup>b</sup>	8,70±0,69 <sup>bc</sup>
<b>AO</b>	2,46±0,11 <sup>a</sup>	2,13±0,94 <sup>d</sup>	4,7±0,54 <sup>c</sup>	8,24±0,60 <sup>d</sup>
<b>JA</b>	2,14±0,10 <sup>d</sup>	2,20±0,12 <sup>c</sup>	5,0±0,66 <sup>c</sup>	7,46±0,70 <sup>f</sup>
<b>JB</b>	2,25±0,12 <sup>c</sup>	2,54±0,16 <sup>a</sup>	6,35±1,19 <sup>ab</sup>	9,05±0,94 <sup>b</sup>
<b>Promedio</b>	2,32±0,18	2,30±0,27	4,91±1,48	8,53±1,07

LS (Seed length), DS (Seed diameter), PS (Seed weight), VS (Seed volume). Values with unequal letters are significantly different ( $P < 0.05$ ) for Tukey's HSD test. PM1 (Altos de Palma Mocha tree 1), PM2 (Altos de Palma Mocha tree 2), PM3 (Altos de Palma Mocha tree 3), RA (Rolando Arriba), RB (Rolando Abajo), AO (Armando Osorio), JA (Syringe Arriba), JB (Syringe Abajo)

The seeds averaged 8,53 cm<sup>3</sup> of volume, being the ones coming from Rolando Arriba, Jeringa Abajo and Rolando Abajo the biggest ones; in this case it can be inferred that these could present difficulties with the predation by animals and microorganisms, hindering the potential of regeneration of seedlings and youngsters in natural conditions.

### Germination and vigour of *Juglans jamaicensis* seeds

*Juglans jamaicensis* seeds showed significant differences in germination potential, depending on the parent tree. Seventy-three percent of them were able to originate a new plant (Table 2).

The high germination capacity is attributed to the use of mature seeds placed to germinate immediately so as not to affect the germination process. The fundamental cause of the seeds not germinated was the process of putrefaction suffered as a result of the excess of humidity caused by abundant rains in the period of trial.

**Table 2.**- Average germination capacity and time to start germination of the seeds of eight *Juglans jamaicensis* trees in the Parque Nacional Turquino

Árbol progenitor	CG (%)	t0 (semanas)
<b>PM 1</b>	97± 4 <sup>a</sup>	4,07±0,9 <sup>a</sup>
<b>PM 2</b>	86±2 <sup>ab</sup>	4,25±1,5 <sup>a</sup>
<b>PM 3</b>	72±7 <sup>bc</sup>	4,32±0,4 <sup>a</sup>
<b>RA</b>	80±14 <sup>ab</sup>	5,21±1,8 <sup>a</sup>
<b>RB</b>	70±8 <sup>bc</sup>	5,50±2,1 <sup>a</sup>
<b>AO</b>	72±17 <sup>bc</sup>	7,96±0,8 <sup>a</sup>
<b>JA</b>	54±7 <sup>c</sup>	4,25±2,5 <sup>a</sup>
<b>JB</b>	54±2 <sup>c</sup>	6,14±1,0 <sup>a</sup>
<b>Promedio</b>	73±16	5,21±1,8

CG (mean germination capacity ± standard deviation), t0 (germination start time), Values with unequal letters are significantly different ( $P < 0.05$ ) for Tukey's HSD test. PM1 (Altos de Palma Mocha tree 1), PM2 (Altos de Palma Mocha tree 2), PM3 (Altos de Palma Mocha tree 3), RA (Rolando Arriba), RB (Rolando Abajo), AO (Armando Osorio), JA (Jeringa Arriba).

The germinative potential obtained exceeds those reported in research carried out with the species: 50 % (Álvarez *et al.*, 2006) and 54,7 % (Rodríguez and Aguilar, 2013), this is basically due to the fact that the seeds were collected at different times and places.

The beginning of germination (t0), on the other hand, did not show significant differences between the parent trees and on average the seeds began to germinate five weeks (35 days) after sowing and finished two weeks later.

The beginning of germination was inserted in the period reported by Álvarez *et al.*, (2006) for the species (11-53 days), but differs from the 41 days indicated by Rodríguez and Aguilar (2013) respectively. The differences may respond to the physiological state, health and seed management.

In table 3, there are significant differences between the speed of germination of the seeds (t50 %, IVG) and the progenitor trees, and the superiority of the trees located in Palma Mocha with respect to the remaining trees was expressed.

Regarding the t50 % parameter, the trees of Palma Mocha, Rolando and Jeringa stand out, whose seeds reach the threshold of 50 % germination between five and seven weeks, being the first, the fastest to germinate, while the seeds of Armando Osorio's tree showed greater slowness of the germination process, so they needed more time to reach 50 % germination (around two months). In general, 50 % of the seeds germinated after seven weeks.

The rate of germination reaffirmed the above. The seeds with the highest germination rate were those of the first two Palma Mocha trees, while those of the UZC Santo Domingo had the lowest germination rate, the rest of the germination rate of the seeds showed intermediate values (0.41 - 0.37). On average the seeds germinate at a rate of 0.40 per week, so it is considered erratic germination seed.

**Table 3.-** Average germination speed and vigour of *Juglans jamaicensis* seeds

Árbol progenitor	t50% (semanas)	IVG	IC
<b>PM 1</b>	5,79±0,9 <sup>b</sup>	0,61±0,1 <sup>a</sup>	0,77±0,4 <sup>a</sup>
<b>PM 2</b>	6,11±1,8 <sup>b</sup>	0,52±0,2 <sup>ab</sup>	0,63±0,4 <sup>ab</sup>
<b>PM 3</b>	6,39±0,9 <sup>ab</sup>	0,41±0,1 <sup>abc</sup>	0,28±0,05 <sup>bcd</sup>
<b>RA</b>	7,29±1,4 <sup>ab</sup>	0,42±0,1 <sup>abc</sup>	0,47±0,1 <sup>abc</sup>
<b>RB</b>	7,14±1,3 <sup>ab</sup>	0,37±0,1 <sup>abc</sup>	0,30±0,1 <sup>bcd</sup>
<b>AO</b>	9,71±0,4 <sup>a</sup>	0,27±0,1 <sup>c</sup>	0,20±0,1 <sup>d</sup>
<b>JA</b>	6,71±1,9 <sup>ab</sup>	0,33±0,1 <sup>bc</sup>	0,21±0,1 <sup>cd</sup>
<b>JB</b>	7,71±1,1 <sup>ab</sup>	0,26±0,03 <sup>c</sup>	0,17±0,04 <sup>d</sup>
<b>Promedio</b>	7,11±1,6	0,40±0,1	0,38±0,3

t50 % (time when 50% germination is reached), IVG (germination speed index (germinated seeds/day)), IC (Czabator index), Values with unequal letters are significantly different ( $P < 0.05$ ) for Tukey's HSD test. PM1 (Altos de Palma Mocha tree 1), PM2 (Altos de Palma Mocha tree 2), PM3 (Altos de Palma Mocha tree 3), RA (Rolando Arriba), RB (Rolando Abajo), AO (Armando Osorio), JA (Jeringa Arriba), JB (Jeringa Abajo).

On the other hand, the vigour of the seeds revealed that those of the trees coming from the UZC Santo Domingo differ significantly from the remaining group of trees studied, as well as that the greatest vigour potential was exhibited by the seeds of the trees of the UZC La Platica, standing out the first two of Palma Mocha and the tree of Rolando Arriba.

*Juglans jamaicensis* seeds reached an average vigor index of 0.38 similar to that found by López and Piedrahita (1999) for *Juglans neotropica* which ranged between 0.30 and 0.43 during the application of pregerminative treatments to reduce the germination period of the seeds of this Latin American mountain species.

The results obtained from the speed index and the vigour of the seeds may influence the maintenance of the species under natural conditions, given that these parameters are important factors for forest species (López and Piedrahita, 1999) due to the high competition that occurs in the early stages of growth.

Also, a series of anomalies (Figure 1) in the plants were found in the nursery. Among the anomalies determined were the presence of albino plants in Rolando Arriba (one plant) and Rolando Abajo (two plants), plants with deformed roots (two plants in RB), stems with strong curvature (four plants in RA and three in RB), multiple stems (three in RA, five in RB and two in JB) and deformation in the roots.



**Fig. 1.-** Anomalies observed in the offspring of *Juglans jamaicensis*

Similar results were reported by Brosi, (2010) and Kimura *et al.*, (2012) with *Juglans cinerea* L. and *Juglans ailantifolia* in Japan and North America respectively, in which phenotypic alterations were found in the leaves as a result of self-pollination or depression by autocrossing. This may constitute an inbreeding depression alert since the species is monoecious, anemophilous, dicogamous and entirely self-compatible.

The seed structure of *Juglans jamaicensis* is an adaptation of the trees to the environment in which they develop as a response to environmental variation.

*Juglans jamaicensis* seeds showed a high germination potential ( $73\pm16\%$ ), low germination speed ( $0.40\pm0.1$ ) and average vigour ( $0.38\pm0.3$ ) as well as anomalies in the offspring, which are determining indicators in the potential decrease of seedling abundance in the forest.

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