



EFFECT OF CONSERVATION TEMPERATURE ON *CAVANILLESIA* *PLATANIFOLIA* SEED VIABILITY

EL EFECTO DE LA TEMPERATURA DE CONSERVACIÓN SOBRE LA VIABILIDAD DE LAS SEMILLAS DEL PIJÍO *CAVANILLESIA PLATANIFOLIA*

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Abstract

Cavanillesia platanifolia is a tree species distributed in the tropical dry forest ecosystem threatened by the fragmentation and loss of its habitat. Reforestation is thus to be considered for its conservation. The *ex situ* production of young individuals is key for any reforestation plan. In this study, we compare the recruitment capacity in nursery of two seed conservation treatments: cold conservation *vs.* conservation at ambient temperature. The emergency rate increased and the seedlings grew more vigorous in cold-preserved seeds. In addition, seedling mortality was significantly reduced in cold-preserved seeds, which we believe is due to a slowdown in seed ageing and a decrease in pathogenic activity. This easily replicable and economic nursery cultivation methodology can be incorporated into the potential reforestation plans of *C. platanifolia*, as well as to other threatened plant species of the neotropic with seeds of similar characteristics.

Keywords: Tropical dry forest, seed conservation, Chocó-Darién, seedling recruitment, Malvaceae-Bombacaceae.

Resumen

Cavanillesia platanifolia es una especie de árbol distribuido en el ecosistema de bosque seco tropical que se encuentra amenazado por la fragmentación y pérdida de su hábitat. La reforestación es una actividad a considerar para su

conservación, y para ello es clave optimizar la producción de individuos jóvenes *ex situ*. En el presente estudio comparamos la capacidad de reclutamiento en vivero de dos tratamientos de conservación de las semillas: conservación en frío vs. conservación a temperatura ambiente. La tasa de emergencia se incrementó y las plántulas crecieron más vigorosas en las semillas conservadas en frío. Además, mediante la conservación en frío de las semillas se redujo notablemente la mortalidad de las plántulas, lo cual creemos que es debido a una ralentización del envejecimiento de las semillas y a la disminución de la actividad patogénica. Esta fácilmente replicable y económica metodología de reproducción en vivero puede ser incorporada a los potenciales planes de reforestación de *C. platanifolia*, así como a otras especies vegetales amenazadas del neotrópico con semillas de similares características.

Palabras clave: Bosque seco tropical, conservación de semillas, Chocó-Darién, germinación de semillas, Malvaceae-Bombacaceae.

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1 Introduction

The Neotropical dry forest stands out for its size, biodiversity, and ecosystem services (Calvo-Rodriguez et al., 2017; Hubbell, 1979). These forests have been subjected to significant anthropogenic impact over the past decades (Aguirre et al., 2017). Particularly in Ecuador, a Neotropical country with the highest number of plant species classified as at least vulnerable (The International Union for Conservation of Nature)(IUCN, 2019), it is estimated that 50% of the dry forest area has disappeared in recent decades due to human activities (Aguirre et al., 2005), mainly as a result of deforestation and land conversion to agricultural and livestock purposes (Madriñán, 2014; Aguirre et al., 2017; Aguirre and Kvist, 2009). The impact these forests are subjected to contrasts with their status as one of the least studied ecosystems across various ecological scales (Aguirre et al., 2017). To identify, establish, and maintain restoration and conservation actions, it is therefore necessary to expand the biological knowledge of at least the most significant plant species (Aguirre et al., 2006).

One of the most significant species in the Ecuadorian dry forest is *Cavanillesia platanifolia* (Humb. Bonpl.) Kunth (Aguirre et al., 2017; Espinosa et al., 2011), commonly known in Central and South America as mocundo de Cartagena, bongo/a, macondo, cuipo, güipo, hameli, hamelí, pretino, pigio, or pijío. Its distribution mainly lies within the tropical dry forests of the Chocó-Darién corridor, from Panama to northern Peru, but it is also present in moist forests.

This tree, belonging to the Malvaceae-Bombacaceae family, can exceed 40 meters in height (Figure 1A), and in Ecuador, it plays a notable role as a habitat generator, among others, for the nesting of the Guayaquil macaw *Ara ambigua guayaquilensis* (López-Lanús and Socola, 2000), and for the nutritional contribution of its seed, which is edible and a resource for local fauna (Adler, 1995; Madriñán, 2014). Its significance at a landscape and structural level is also noteworthy (Aguirre, 2012). It is utilized as a timber resource by local communities in various countries, such as Panama (Correa et al., 2004), although there is no record of this in Ecuador (Madriñán, 2014). The species has been declared globally threatened in Central America (IUCN,

2019). In Colombia, where it has been indexed as one of the most important species of the tropical dry forest (Ruiz and Saab, 2020), it is considered endangered (IUCN, 2019). Its potential distribution in this country may not be severely threatened by climate change, although it is expected that it could decimate its populations (Aguirre et al., 2017).

Many characteristics are common among the different species of the genus, such as sciophily; the seedlings (Figure 1B) and juveniles grow in places where solar radiation is scarce (Vieira et al., 2007; Montalvo et al., 2013). Generally, these species are characterized by their low density (Vieira and Scariot, 2006b; Vieira et al., 2008; Melo-Júnior et al., 2015), rapid growth (Condit et al., 1993), and drought tolerance during the dry season (Wolfe, 2017). In September-October, they produce their large, fleshy fruits, containing an anemochorous seed with an outer shell that includes soft mucilage (Vieira and Scariot, 2006b). The seed traits have been studied primarily in *C. arborea*. The seeds of this species weigh around 8g fresh, while the dry weight is about 1g (Vieira and Scariot, 2006b; Romero-Saritama, 2016). Over time, the seeds lose viability (Walters et al., 2005) and are sensitive to desiccation (Vieira and Scariot, 2006b; Vieira et al., 2007, 2008; Lima et al., 2008). As with many species of the tropical dry forest, the low humidity keeps the seeds dormant, which can be interrupted during the rainy season, activating the embryo for germination (Debeaujon et al., 2000).

When individuals of *C. platanifolia* have been sampled in the dry forests of Ecuador for conservation and restoration plans, the most relevant finding is the low presence of seedlings and juveniles, indicating a very low rate of natural regeneration (Villalba-Briones, personal observation). The absence of young individuals of *C. platanifolia* not only occurs near adult individuals, often related to high density-dependent mortality due to pathogens, seed predators, and/or herbivory under the maternal canopy (Alvarez-Loayza and Terborgh, 2011; Comita et al., 2014; Xu et al., 2015), but is consistent across the forests.

In other species of Malvaceae-Bombacaceae from the Neotropics, such as *Pachira quinata* (Jacq.) Alverson, low rates of natural regeneration have also been observed, attributed to decreased fruit

production, intense fruit predation, and seedling mortality due to pathogens (Castellanos and Stevenson, 2011).

Regarding seed predation, it is known that in other species of the genus *Cavanillesia*, such as *C. arborea* (Willd.) Schum. and *C. chicamocha* Alonso, their seeds are intensely predated both pre-dispersively (various insects and parrots of the family Psittacidae) and post-dispersively (rodents and other mammals), and seed predation can significantly increase with silvopastoral activity (Vieira and Scariot, 2006a; Vieira et al., 2007; Díaz-Pérez et al., 2011; Souza-Silva et al., 2015). While silvopas-

toral activity is not as intense in the dry forests of Ecuador (Aguirre, 2012), the rate of seed predation, particularly by rodents may have increased due to the continued loss of carnivorous mammals (Catterall, 2018) and defaunation of large herbivores due to hunting pressure (Rosin and Poulsen, 2016). More importantly, as observed for *C. arborea* and *C. chicamocha*, other anthropogenic activities such as forest fragmentation and land conversion may be intensely reducing the emergence and recruitment of *C. platanifolia* seeds (Vieira and Scariot, 2006b; Vieira et al., 2007; Melo-Júnior et al., 2015; Souza-Silva et al., 2015).

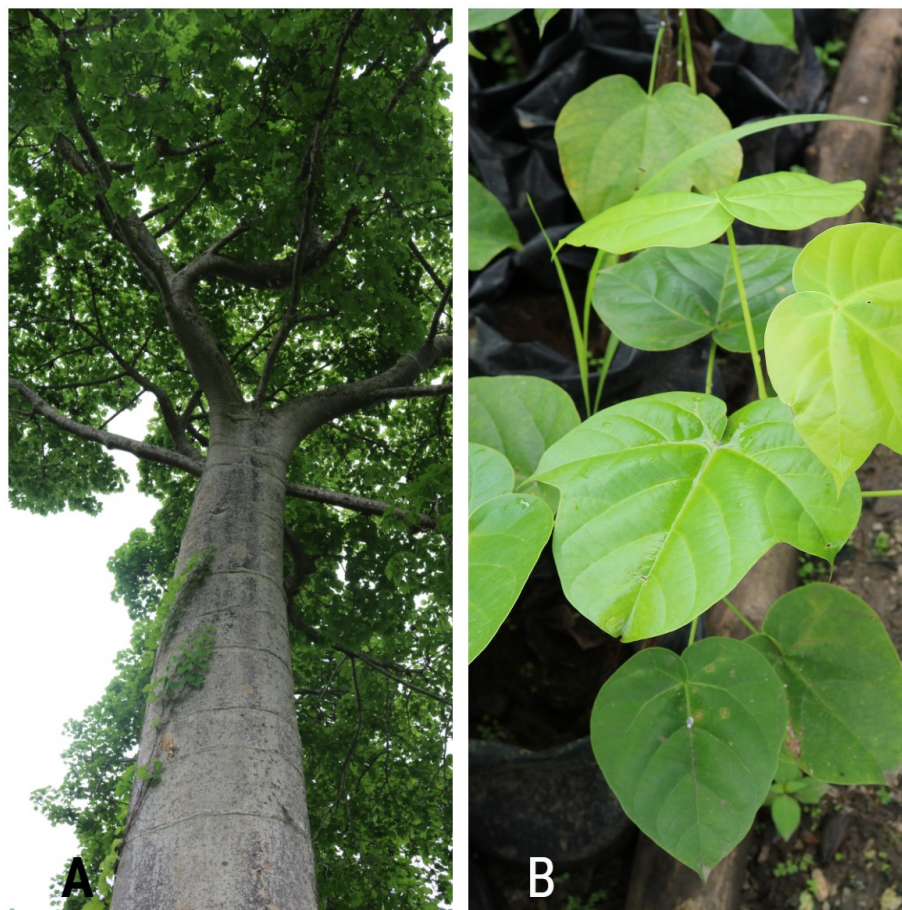


Figure 1. *Cavanillesia platanifolia* (Humb. & Bonpl.) Kunth (A) Specimen from the sampling point in the Guayas province (Ecuador), with its characteristic transverse rings on the trunk. (B) Seedling in nursery with its simple and trilobed leaves.

Understanding fundamental concepts in biology, such as seed viability and recruitment capacity, represents a crucial area of investigation in the realm of species conservation. Within tropical ecosystems, seeds play a pivotal role in the reproductive process, with their persistence, survival, and emergence serving as key determinants for the generation of seedlings and young trees pivotal to restoration initiatives (Vieira and Scariot, 2006a; Sarmiento et al., 2017). Particularly, the effective conservation of seeds is imperative for the planning and execution of species propagation within a nursery setting, as a strategy for ex situ conservation (Becerra-Vázquez et al., 2018). This study examines the hypothesis that cold storage of *C. platanifolia* seeds may enhance their germination, survival rates, and overall yield.

2 Materials and Methods

2.1 Study area

The dry forest in Ecuador is located on the western flank of the Andes, in the provinces of Esmeraldas, Manabí, Guayas, El Oro, and Loja (Aguirre et al., 2017). *Cavanillesia platanifolia* is commonly found in these forests below 800 meters above sea level (Jørgensen and León-Yanes, 1999; Aguirre et al., 2006). According to Espinosa et al. (2011), it is distributed across the provinces of El Oro, Loja, Manabí, Guayas, and in the Tumbesian dry forest, as well as in the province of Esmeraldas (Aguirre et al., 2017).

For this study, seeds of *C. platanifolia* were collected from Hacienda Pabeclar, located in the Chongón Protective Forest (2° 7' 13.97"S, 80° 4' 57.78"W; Guayas province, Figure 2). This region, bordering the Chongón-Colonche Mountain range and being part of the Chocó-Darién corridor on the coast of Ecuador, is a priority conservation target (Mittermeier et al., 2011). In the forest area, agricultural activity is increasing, mainly due to the expanding cultivation of corn (Figure 2). The seed collection took place in November 2017, in an area of approximately 6000 m², selecting those from the ground that showed no signs of predation (Bonfil-Sanders et al., 2008; Orantes-García et al., 2013). Within the collection area, the individuals (N=6) of *C. platanifolia* were characterized, which in this area had a height circumference (CBH) at 1.30 m from the ground of 321 cm to 570 cm and a height of 25 to 37 m, measu-

red using the Range Finder technique (Wing et al., 2004).

2.2 Conservation methodology, nursery methodology, seed sowing, and monitoring of seedling emergence and performance

2.2.1 Pilot test

A greenhouse pilot study with 187 seeds (starting the seed hydration on February 28, 2018) was conducted to verify the viability of the collected seeds. Prior to planting, from the previously mentioned collection, the seeds were stored in a dark, enclosed space at room temperature (21.3°C- 26.8°C) for 3 months. Afterwards, the seeds underwent a physical scarification process, and were then soaked for 48 hours. According to Trujillo (1995), this hydration should not be less than two hours, but also not exceed 48 hours to avoid possible fermentation.

After hydration, seeds were arranged in 1m² x 30 cm high squares with 2 cm between them. Due to the high mortality of *C. platanifolia* seedlings caused by pathogens (Villalba-Briones et al., personal observation), a silica-rich sand substrate with a fungicidal effect was used. The squares were placed in a nursery (greenhouse) covered with a saran mesh, which reduces solar radiation by 80% and favors the emergence of seeds from other species of the genus (Vieira et al., 2008). From that point, the squares were watered in the nursery 5 days a week for 10 minutes by misting. From the time of planting, and over 1 month, we measured the cumulative emergence rate, which was 89.3%.

2.2.2 Response test to conservation conditions

With seeds of the same origin and collection date, we proceeded with the experimental trial, with a longer storage time than in the pilot test. For this experiment, first, the collected seeds of *C. platanifolia* were stored dry in a closed space protected from external environmental elements for a total of 3 months (November 2017 to February 2018), after which they were randomly assigned to 2 treatments with 55 seeds per treatment. For the first treatment, the seeds were stored for an additional 6 months at room temperature (21.3- 26.8°C) and in darkness.

For the second treatment, the seeds were stored in a refrigerator (4.3- 7.6°C) for the same period. Through the cold storage conservation treatment, we aimed to verify if it favors the conservation of seeds maintaining their viability. This has been suggested

by several authors (Becerra-Vázquez et al., 2018; Li and Pritchard, 2009), although high intraspecific variability has also been observed (Posada et al., 2014; Trujillo, 1995).

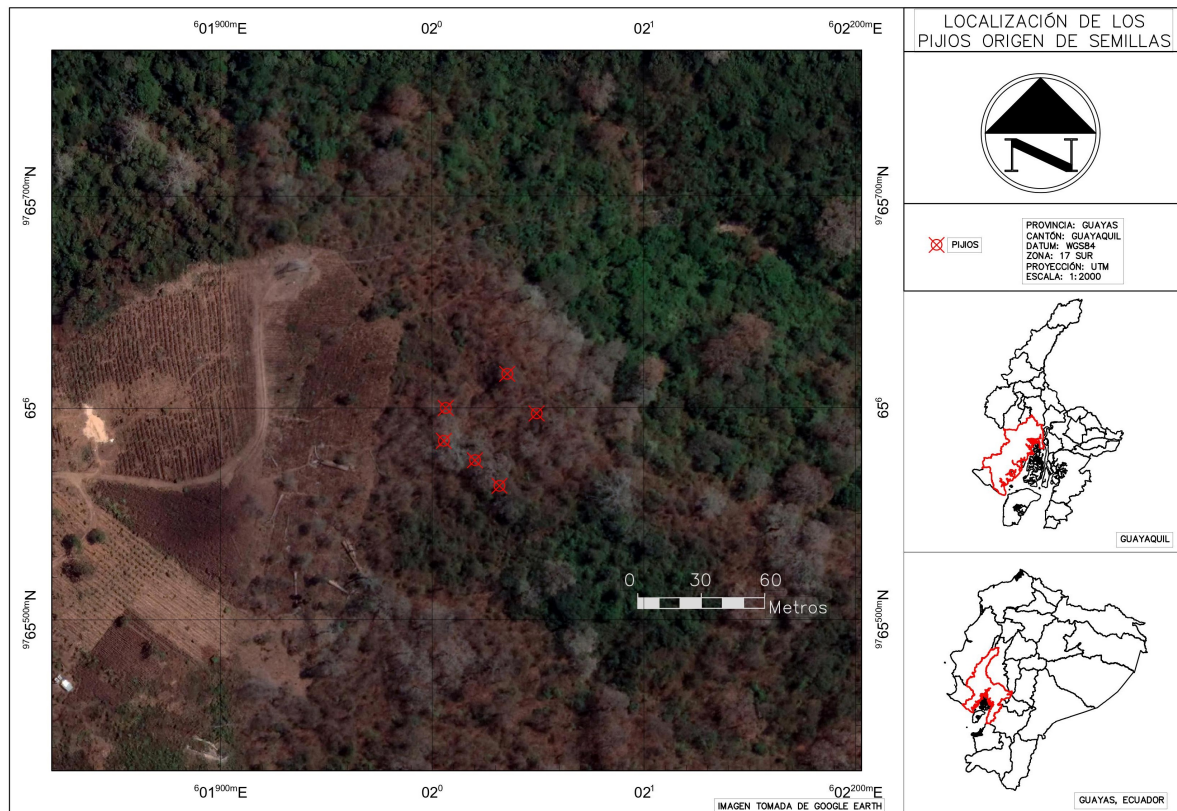


Figure 2. Map representing the seed collection area of *Cavanillesia platanifolia* in the Guayas province (Ecuador), marking the location of the characterized trees. Map developed using ArcGIS 10.3.

This observed intraspecific variability is largely due to the morphological and physiological characteristics of the seeds, such as their water content and thermotolerance (Kranner et al., 2010; Sasaki, 2008). For our experimental trial, we opted for refrigerator conservation as it is cost-effective and easily reproducible, considering that conservation in liquid nitrogen (-196°C) does not significantly increase emergence in other species like *C. arborea* (Lima et al., 2008). After 6 months of treatment and prior to planting, the seeds underwent a physical scarification process, and were then soaked for 24 hours. After hydration, we proceeded to sow and cultivate in the nursery (greenhouse) in the same

way as described for the pilot trial. At the same time, the seed development progression was photographically recorded in a glass container adapted for image collection (Figure 3).

From the moment of sowing and over 1 month, we measured the cumulative emergence rate and mortality of the emerged seedlings. From these variables, we also estimated recruitment success (emergence + survival). In turn, we measured the growth (height) of the seedlings at 14 and 25 days after sowing (see example of seedling in Figure 4). Throughout the process, environmental parameters of temperature, relative humidity, and dew point

were measured. The measurements were taken with a thermohygrometer, taking 3 measures per square/day to then make the daily average. The measurements were homogeneous over time, with a varia-

tion of 3°C in temperature (27.3- 30.3°C) and 2.8°C in dew point (21.7- 24.5°C). Regarding relative humidity, it did not fluctuate beyond 25% between the different measurements (65.1- 85.3%).

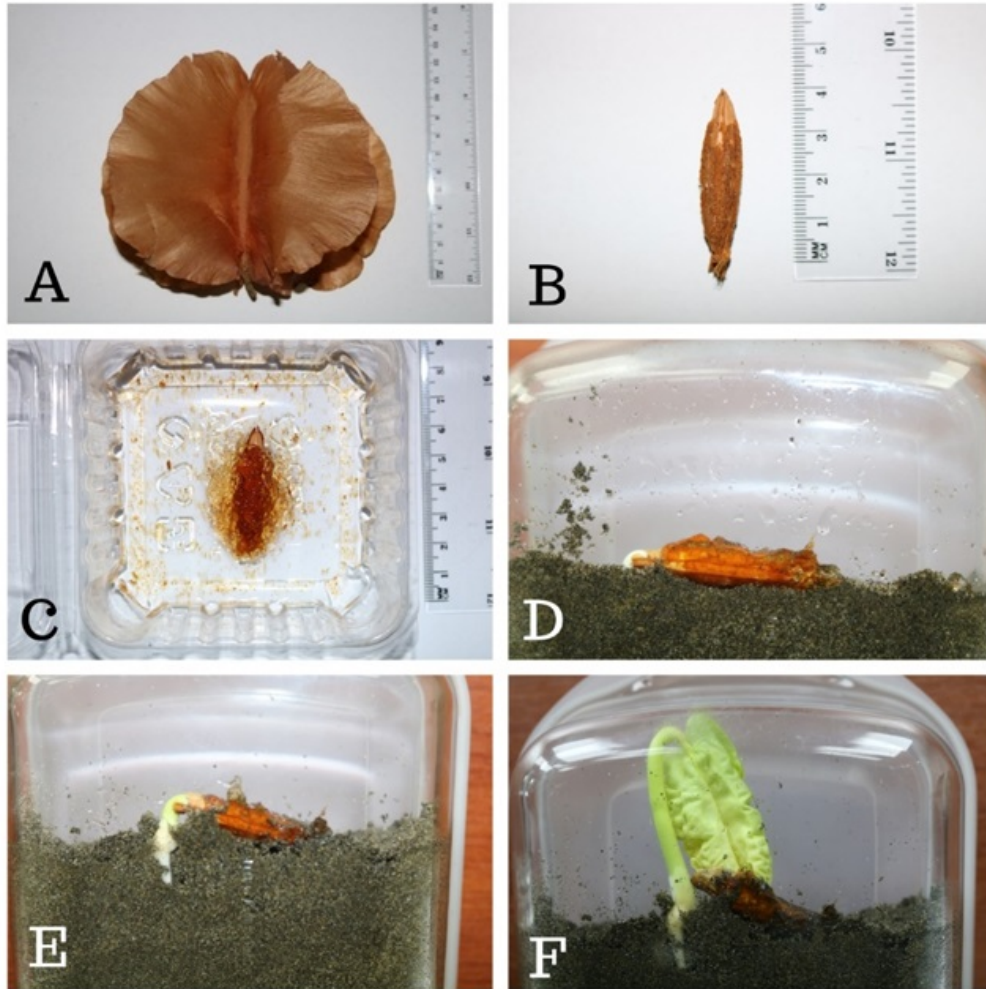


Figure 3. *Cavanillesia platanifolia* (Humb. & Bonpl.) Kunth. (A) Collected seed with its 4 wings for anemochorous dispersion. (B) Seed once dehulled. (C) Seed hydrated at 24 hours. (D) Positioning in sand of a seed for germination. (E) Seed germinating at 48 hours. (F) Emergence of the seedling.

2.3 Statistical analysis

We analyzed the effect of both treatments (cold storage vs. room temperature storage) on seed emergence, mortality, and recruitment success using generalized linear models with a binomial distribution. Regarding the effect of the treatments on the growth (height) of the seedlings at both temporal

measurements (14 and 25 days after sowing), we evaluated whether the normality parameters were met. Quantile-Quantile plots determined that the data fitted well to a normal distribution, and the Shapiro-Wilk normality test determined that the null hypothesis of normality assumption should not be rejected in both cases. Therefore, the height of the seedlings could be analyzed using a general linear

model (one-way ANOVA). The analyses were performed using the statistical software R 3.5.2 (R Core

Team, 2017), through the nlme (Pinheiro et al., 2011) and lme4 (Bates et al., 2014) packages.



Figure 4. *Cavanillesia platanifolia* (Humb. & Bonpl.) Kunth. Axonomorphic root and simple cuneate leaves of four-day-old seedling.

3 Results and Discussion

In the experimental trial, seeds preserved in cold storage exhibited a 10 % higher emergence rate than those stored at room temperature (98.18 ± 1.81 vs. 89.09 ± 4.24 %, $\chi^2 = 4.20$, $P = 0.040$; Figure 5A) and, furthermore, the mortality rate with cold storage was 317% lower once emerged (16.66 ± 5.11 vs. 71.42 ± 6.52 %, $\chi^2 = 33.30$, $P < 0.0001$; Figure 5B). Mortality was severely influenced by bacterial and especially fungal infections that affected the endosperm and cotyledons. The recruitment success of the seeds, considering both emergence and survival, was 228% higher in seeds preserved in cold compared to those kept at room temperature (81.81 ± 5.24 vs. 25.45 ± 5.92 %, $\chi^2 = 37.35$, $P < 0.0001$; Figure 5C).

In addition to higher recruitment, seedlings from seeds stored in cold showed greater growth during the follow-up conducted the month after sowing. Thus, seedlings emerged from seeds stored in cold were 45 % taller than those stored at room temperature at fourteen days (8.60 ± 0.42 vs. 5.92 ± 0.73 cm, $F_{1,58} = 9.53$, $P = 0.003$; Figure 5D), and 28 % taller at twenty-five days (17.10 ± 0.55 vs. $13.29 \pm$

1.28 cm, $F_{1,57} = 9.74$, $P = 0.002$; Figure 5D).

Various factors are decimating the populations of *Cavanillesia platanifolia* in the Neotropics. The loss and fragmentation of forest areas and their logging are formed by the recently observed low natural regeneration in the dry forests of Ecuador. This could be due to a decrease in the number of individuals and lower production of fruits and seeds, a higher seed predation rate, an increase in seed desiccation and/or in seedling mortality due to environmental factors. Thus, the establishment of young individuals may be compromised by increasing physiological stress due to the ever-greater seasonal climate fluctuations. This situation could worsen in the future, so it would be advisable to fully study the life cycle of *C. platanifolia* up to its adult stage and determine the influence of various biotic and abiotic factors on its biological effectiveness. It cannot be ignored that seed viability may also be decreasing due to lower rates of cross-pollination (Hamrick and Murawski, 1990).

This study demonstrates that cold storage of seeds for six months allowed for the maintenance of their viability compared to seeds stored at

room temperature, increasing the emergence rate. Cold storage would thus maintain the viability of seeds sensitive to desiccation (Normah et al., 2019), as is presumably the case with *C. platanifolia*. Our study shows that cold storage of the seeds for 6 months significantly maintained the seeds' viability. The emergence of seeds stored in cold for six

months (with three previous months of room temperature storage in a closed space) was similar, and even slightly superior to the emergence observed in three-month seeds at room temperature in the same closed space (pilot trial), an effect possibly mediated by the interruption of dormancy due to cold.

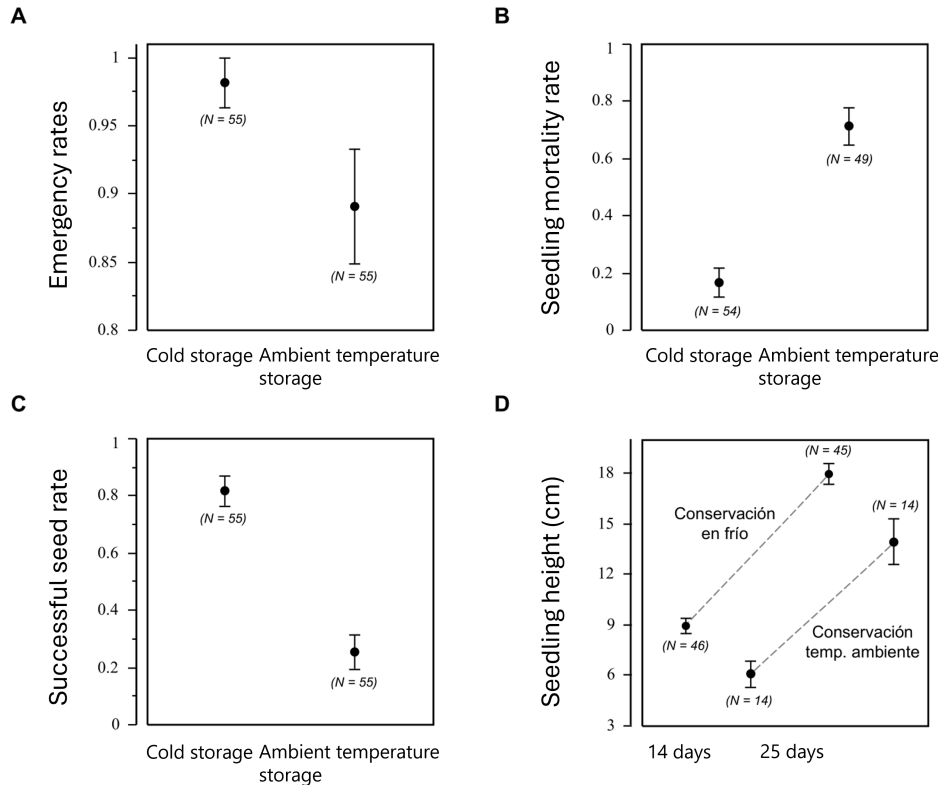


Figure 5. Effect of the seed storage temperature of *Cavanillesia platanifolia* (Humb. Bonpl.) Kunth on the observed variables. **(A)** Emergence rate. **(B)** Seedling mortality rate. **(C)** Recruitment rate (successful seeds). **(D)** Height of the seedlings with different treatments at 14 and 25 days after sowing.

Moreover, it is noteworthy that seedlings from the cold storage treatment grew more vigorously. This type of conservation would limit the adverse effects of high temperatures and water deficit on the seeds, reducing their aging. Through the multicausal phenomenon of aging, the physiological quality of seeds is reduced, inducing structural, compositional, and functional changes, limiting their viability and vigor (Kranter et al., 2010; Li and Pritchard, 2009). However, the most novel and notable result is that the mortality of these seedlings was considerably reduced in seeds stored in cold. Pathogeni-

city is a determining factor in seedling recruitment (Kranter et al., 2010), being one of the major causes of their mortality during their first month of life in tropical forests (Comita et al., 2014).

In these forests, pathogen attack usually has more acute effects on those seedlings that grow in shade (Augspurger and Kelly, 1984), as is the case with *C. platanifolia*. Cold storage, therefore, would not only maintain the vigor of the seeds but also reduce the activity of pathogens, limiting their negative impact on the physiology of the seeds and the es-

tablishment of the seedlings. It is common in tropical forests for seedling mortality due to pathogenic action to also be linked to their density (Alvarez-Loayza and Terborgh, 2011; Augspurger and Kelly, 1984). Our methodology shows that low mortality rates can be achieved even when sowing at high densities (~ 83 % survival in seeds stored in cold, with 2 cm distance between seedlings).

4 Conclusions

We consider that cold storage could decrease mortality by both limiting the weakening caused by aging and slowing down the growth of pathogens, leading to a lower probability of lethal infection. The easily replicable and cost-effective nursery methodology used in this study can be employed for the production of seedlings in the necessary reforestation plans for *C. platanifolia*. Thus, the nursery can be utilized at different sowing times, planning the activation and sowing of the seed kept in cold storage as convenient, thereby optimizing efficiency in seedling production.

This methodology could also be employed in other species of the Malvaceae-Bombacaceae family, as nearly half of the species in this family are listed as at least vulnerable (IUCN, 2019). The methodology could similarly be tested on other important and threatened species from the Chocó-Darién with seeds of similar characteristics, among which are *Quercus humboldtii* Bonpl. (Fagaceae), *Prioria copaifera* Griseb. (Fabaceae), *Caryocar amygdaliferum* Mutis (Caryocaraceae), and *Anacardium excelsum* (Bertero Balb. ex Kunth) Skeels (Anacardiaceae).

Author contribution

R.V.B.: Conceptualization, visualization, research, resources, methodology, formal analysis, project administration, data collection, data processing, supervision, writing-original draft, writing-review and editing. E.R.J.: Visualization, resources, methodology, project administration, data collection, supervision, writing-review and editing. A.R.L.: Research, methodology, resources, validation. M.A.B.: Research, formal analysis, data curation, validation, writing-review and editing.

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