Effects of Hydrogel Use in *Cariniana pyriformis* (Lecythidaceae) Seedlings Under Different Water Regimes

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**Abstract**

The effect of hydrogel (0, 3, 6, and 9 g per seedling) on the survival and growth of *Cariniana pyriformis* seedlings under different water regimes (absence of irrigation, 60% and 100% field capacity) in a sandy loam soil was assessed. The experiment was carried out in an agricultural nursery with drip irrigation, implementing a completely randomized block design in a split-plot scheme. After 20 weeks of evaluation in both treatments with irrigation, the maximum dose showed a slight increase (9%) in stem diameter and seedling height compared with the control treatment, although the aerial and root dry biomass did not show differences. For the treatment without irrigation, the survival had a linear response with increasing doses, from 24% (0 g per seedling) to 65% (9 g per seedling). *C. pyriformis* responds positively to hydrogel when a severe water deficit occurs.

**Keywords:** Colombian mahogany, hydro retainer polymer, reforestation, water stress.

1. **INTRODUCTION AND OBJECTIVES**

Currently, the policies and regulations that govern the activities related with the use of wood in Colombia have been insufficient to counteract illegality, indiscriminate use, and the scarce organization in the commercialization of timber species (PROFOR, 2017). Moreover, the lack of silvicultural knowledge places native species at a disadvantage compared to exotic species that have traditionally been cultivated in the country, such as *Pinus* spp., *Eucalyptus* spp., *Acacia mangium* Willd., *Tectona grandis* L.f. and *Gmelina arborea* Roxb. ex Sm. (PROFOR, 2017).

The abarco (*Cariniana pyriformis* Miers), commercially known as Colombian mahogany, is characterized by its arborescent habit, reaching up to 50 m tall, simple leaves of crenate margins, pink petals, and turbinated fruits (Prance & Mori, 1979; Mori et al., 2019). In addition, due to the properties of its wood, such as hardness and resistance to weathering, the abarco is of high economic value and has been widely exploited, which has led it to be categorized locally as Critically Endangered (CR) (Salinas & Cárdenas, 2007; Cárdenas et al., 2015), and globally as Near Threatened (NT) (IUCN, 2019). In this regard, its conservation in agroforestry systems with cocoa has been promoted as a strategy to improve the production of this crop (Agudelo-Castañeda et al., 2018; Suarez et al., 2018). *C. pyriformis* is distributed from northwestern South America to Venezuela (Prance & Mori, 1979; Mori et al., 2019). In Colombia, the species grows in humid tropical forests between 0 and 800 m of altitude, from the north of the biogeographic Chocó region to the Catatumbo moist forests (Celis, 2016; Mori et al., 2019). These ecoregions are characterized by the absence of a defined dry season and with more than 3,500 mm of annual precipitation (Moreno & Del Valle, 2015). Furthermore, it grows in the inter-Andean valleys of the Magdalena River with a bimodal precipitation regime (Pelaez et al., 2018). However, its presence has also been reported in the tropical dry forest of the Caribbean coast, a biome characterized by having an annual rainfall of less than 2,000 mm and one or two periods of drought lasting more than three months (accumulated rainfall <300 mm) (Pizano & García, 2014; Espitia et al., 2017).

Hydrogels (i.e., super-absorbent polymers), which have a water absorption capacity of up to 300 times their volume, releasing the water in a controlled manner to the seedlings, have been used for 30 years or more in agriculture and the timber industry (Crous,
Due to a higher availability of water and nutrients in the soil, hydrogels increase the growth and survival of seedlings as reported for *Eucalyptus dunnii* Maiden (Navroski et al., 2015), *Coffea arabica* L. (Souza et al., 2016) and *Pinus patula* Schiede ex Schltldl & Cham (Madhangingany et al., 2018), and species from the Cerrado in Brazil (i. e. *Enterolobium contortisiliquum* (Vell.) Morong, Fonseca et al., 2017; Pontes Filho et al., 2018). However, the time and application methods of the hydrogel, as well as the irrigation frequency, polymer type, texture and other soil physical properties have an influence on the optimal doses and affect its performance depending on the species (Crous, 2017).

*C. pyriformis* is a semi-deciduous species (Pelaez et al., 2018) that is probably adapted to drought, considering the climates where it grows. However, several factors such as the non-uniformity in the growth of the plantation and the costs associated to dead seedling replacement, limit conservation and commercial reforestation projects (Barbosa et al., 2013; Fonseca et al., 2017). Accordingly, the aim of this study was to evaluate the survival and growth of *C. pyriformis* seedlings using hydrogel under different water regimes.

### 2. MATERIALS AND METHODS

#### 2.1. Plant material

The study was carried out during the period from April to August of 2019 in the municipality of Rionegro, department of Santander, Colombia (7° 22' 10'' N, 73°10' 39'' W; 550 m of altitude), corresponding to a humid tropical forest. Seeds of *C. pyriformis* were purchased from the company Semicol, originally obtained from the municipality of San Luis, Antioquia. Once the seedlings reached one month of age, these were transplanted to conical tubes (8 cm diameter x 25 cm height) and planted in a substrate composed of 60% soil + 20 % river sand + 20% commercial compost obtained from composting poultry manure. The seedlings were maintained for five months in agricultural nursery covered with black monofilament screen and mesh to offer 65% of shading.

#### 2.2. Soil characterization and hydrogel application

Seedlings with an average of 60 cm in height and 5 mm in stem diameter were transplanted into plastic bags (15 cm diameter x 30 cm height), at a density of 12 seedlings per m². They were placed in an agricultural nursery with 50% shading given by a plastic coverage (Agrofrio X) and drip irrigation to simulate their planting in the field, using an automated timer system and self-compensated drippers with a flow of 1.2 L h⁻¹. The temperature and relative humidity were recorded every 10 minutes using a data logger (CEM, DT-172) with averages of 26 ± 5 °C and 88 ± 5.5%, respectively.

Initially, the bags were filled up to 70% of their capacity with sandy loam soil, adding the slow-release fertilizer Basacote Plus 6 M (16-8-12-5; N-P₂O₅-K₂O-S), at a rate of 2 g per liter. They were placed in an agricultural nursery with 50% shading given by a plastic coverage (Agrofrio X) and drip irrigation to simulate their planting in the field, using an automated timer system and self-compensated drippers with a flow of 1.2 L h⁻¹. The temperature and relative humidity were recorded every 10 minutes using a data logger (CEM, DT-172) with averages of 26 ± 5 °C and 88 ± 5.5%, respectively.

| Soil texture (1) | sand (%) | 67.4 |
|------------------|----------|------|
| Silt             | 14.2     |
| Clay             | 18.4     |

**Table 1.** Physical and chemical properties and water retention curve (WRC) of the soil used in the experiment.

| Physical properties | Chemical properties |
|---------------------|---------------------|
| WRC (curve tension kPa) |
| pH (5) | 6.7 |
| E.C (5) | dS m⁻¹ |
| O.M (6) | g 100 g⁻¹ |
| N (7) | % |
| P (8) | mg kg⁻¹ |
| S (9) | 3.6 |
| Fe (9) | 29.8 |
| Ca (10) | cmol (+) kg⁻¹ |
| Mg (10) | 0.7 |
| K (10) | 0.1 |
| Na (10) | 0.14 |

(1) bouyoucos method. (2) undisturbed sample. (3) field capacity was considerate at -10 kPa. (4) permanent wilting point was considerate at -1,500 kPa. (5) pH and electrical conductivity (E.C) at a soil: water ratio of 1:5. (6) organic matter (O.M) by the Walkey and Black method. (7) Kjeldahl by colorimetry. (8) monobasic calcium phosphate. (9) modified Olsen. (10) 1 N ammonium acetate with a pH of 7.
According to the seller, the used hydrogel (Hidrokeeper) contains molecules of acrylamide and potassium acrylate with an absorption capacity 300 times its weight, density of 0.8 g cm\(^{-3}\), usable pH index from 5 to 9, insoluble in water, particle size from 0.2 to 5 mm and a shelf life of five years. Four doses of hydrogel were evaluated (0, 3, 6, and 9 g per seedling). The total amount was hydrated for 72 hours, with 300 ml of water per dry g of the hydrogel. During the transplant and according to the volume per dose, half of the hydrogel was applied to the bottom of the bag and then covered with a layer of soil, and the other half was spread around the stem.

Three water regimes were evaluated according to the irrigation depths, i.e., absence of irrigation, 60%, and 100% field capacity (FC) of soil. Following the transplant, the seedlings without irrigation were kept for 8 days at FC, whereas those of the other water regimes remained 40 days at FC. The soil moisture content was calculated weekly using the weight loss of two plastic bags per water regime, employing an electronic balance (0.1 g). The water volume needed to restore the humidity to 100% and 60% FC was applied every Tuesday and Saturday in three turns (08:00, 11:00 and 16:00 h), according to Equation 1:

\[ Id = FC \times Db \times Bd \times Ab \times Ie \]

Where \(Id\) is the irrigation depth (mm), \(FC\) is the field capacity of soil at -10kPa (% moisture by weight), \(Db\) is the depth of the plastic bag (cm), \(Bd\) is the bulk density of sandy loam soil (g cm\(^{-3}\)), \(Ab\) is the area (cm\(^2\)) of the plastic bag, and \(Ie\) is the drip irrigation system efficiency (90%).

### 2.3. Experimental design and data analysis

A randomized complete block design was adopted in a split-plot scheme with three repetitions of five seedlings each; the main plot corresponded to the three water regimes according to the irrigation depth (absence of irrigation, 60%, and 100% FC of soil) and the subplot corresponded to the four doses of hydrated hydrogel (0, 3, 6 and 9 g per seedling). For the treatment without irrigation, the wilting stages were recorded every week (on Thursday) during 20 weeks (142 days) for each seedling, using a classification based on six visual characteristics (see Table 2; adapted from Engelbrecht & Kursar, 2003), and calculating the duration in days of each of the categories.

| Stage          | Visual characteristics                                      |
|----------------|------------------------------------------------------------|
| 0 Normal       | No signs of wilting or water stress.                        |
| 1 Slightly wilted | Some leaves lose turgidity, but none are rolled or folded. |
| 2 Wilted       | Leaves begin to hang and have lost turgidity. The leaf angle is close to -45° in relation to the horizontal plane. |
| 3 Severely wilted | Beginning of the generalized leaf necrosis (with gray-green to gray-brown areas). |
| 4 Nearly dead  | Most of the leaves are necrotic, some young leaves still green near the midrib. The stem is alive and is distinguished by its color and elasticity. |
| 5 Dead         | Necrosis of all leaves and the stem has lost its elasticity.|

Adapted from Engelbrecht & Kursar (2003).

Two variables were measured biweekly: plant height (H), measured with a flexometer (0.1 cm) from the stem base to the apical meristem, and stem diameter (SD), measured with a digital caliper (0.01 mm). At the end of the evaluation period, the survival percentage was established as 1 for seedlings that were alive, and zero (0) for dead seedlings, i.e., equivalent to a range of 0-100%. Also, four randomly selected seedlings per treatment were used to calculate the dry mass of their aerial organs (DMA) and their dry root mass (DRM). This was carried out in a forced ventilation oven at 65 °C until constant weight. Using these values, the Dickson’s quality index was calculated according to Equation 2 (Dickson et al., 1960):

\[ DQI = \frac{(DMA + DRM)}{((H / SD) + (DMA / DRM))} \]

Where \(DQI\) corresponds to Dickson’s quality index, \(H\) [cm] to the plant height, \(SD\) [mm] to the stem diameter, \(DMA\) [g] to the dry mass of the aerial organs, and \(DRM\) [g] to the dry root mass.

The normality and homoscedasticity of the variance in the data were evaluated according to the Shapiro-Wilk and Levene's test, respectively. When significant differences \((p<0.05)\) were found in the variables DQI, DMA, DRM and duration of the water stress categories, the comparison of means was carried out with Tukey's test. On the contrary, if the assumptions were not fulfilled, the non-parametric Kruskal-Wallis test was performed. The dose interactions for each water regime in the height and stem diameter variables were unfolded when it was significant \((p<0.05)\).
by the regression analysis, evaluating the linear and quadratic regression models. The one with the highest coefficient of determination (R²) and significance found by the F test was selected. The analyses were performed in the statistical program S.A.S, version 9.3.

3. RESULTS AND DISCUSSION

3.1. Plant height and stem diameter

The increase in height and stem diameter over time showed an interaction between the two factors evaluated. In the treatment without irrigation, these variables showed a negative quadratic response for the four hydrogel doses, although with lower coefficient values for the dose of 0 g per seedling (R² = 0.6). The stem diameter reached a maximum on average at 81 days (between 5.81 and 6.07 mm), whereas it was at 105 days for the plant height (between 70.4 and 72.3 cm), thereafter a reduction of these values took place (see Figure 1A and 2A).

On the other hand, in the water regimes of 60% and 100% FC, the variables showed a positive linear trend for all hydrogel doses. After 20 weeks since the transplant, the application of 9 g per seedling increased the values of stem diameter (+ 14%; 7.8 to 8.9 mm) and height (+ 4%; 93.5 to 97.2 cm) compared to the control treatment in the water regime with 60% FC (see Figure 1B and 2B). This slight positive effect of the hydrogel was similar in the water regime with 100% FC for stem diameter (+ 4.1%; 9.0 to 9.4 mm) and height (+ 8%; 100 to 108 cm), respectively (see Figure 1C and 2C).

![Figure 1. Stem diameter (SD) of C. pyriformis seedlings until 20 weeks after transplant at bags subjected to different hydrogel doses according to the water regime applied: in the absence of irrigation (A), 60% FC (B), and 100% FC (C). FC= field capacity of soil.](image-url)

The luminosity of 50% in which the C. pyriformis seedlings were maintained and the fact that the hydrogel has shown higher utility when used in severe water deficit, could have caused the poor differences between irrigation treatments. Another Cariniana species such as C. estrellensis (Raddi) Kuntze, considered as a non-pioneer species that grows in the semi-deciduous forest (Mata Atlântica) in Brazil, demonstrated its tolerance under full sun exposure (Gaburro et al., 2014). Pontes Filho et al. (2018) found that for Enterolobium contortisiliquum (Vell.) Morong, a pioneer tree species of savannas (Cerrado) in Brazil, the hydrogel dose that provided the best growth was lower in seedlings grown under full sun exposure (2 g) compared to those grown in a nursery with 50% shading (3.5 g). The authors argued that higher evapotranspiration under full sun exposure induced lower doses to have a stronger effect. Perhaps C. pyriformis also has a high plasticity and requires a less intense level of shading during the early growth stages, which would be an aspect to be evaluated considering the natural conditions to which the species is adapted in its early development stages.
Effects of Hydrogel Use in Cariniana pyriformis...

3.2. Dry mass of the aerial part, dry root mass and Dickson’s quality index

Only a simple effect could be noticed under the water regimes in DRM, DMA and DQI. In the treatment without irrigation, the biomass reduction compared to the treatments with irrigation was more drastic for the DMA (-73%) than for the DRM (-46%). A typical response to severe water deficit is the reduction of the leaf area through leaf necrosis because the seedling reduces water loss due to evapotranspiration at the expense of photosynthesis, and hence, biomass accumulation (Le Gal et al. 2015).

On the other hand, there were no significant differences in the treatments with irrigation, reaching on average 5.5 g of DRM, 17.5 g of DMA and 5.8 for DQI (see Table 3). As mentioned before, this might be related to the fact that the positive effects of the hydrogel are more noticeable in dry conditions. The absence of an adverse effect between the evaluated doses would indicate that the expansion of the hydrogel did not limit root development in the sandy loam soil.

The lower water availability with the 60% FC treatment did not increase the DRM, which could be due to a response mechanism associated mostly with the physiology of Cariniana pyriformis than with morphological changes due to water stress. Markesteijn & Poorter (2009) evaluated 62 tropical trees and concluded that the species vary strongly in their morphology and biomass translocation related with their...
tolerance to drought and shade after the first year, being the drought-tolerant species those that displayed higher root biomass. In parallel, Cordia trichotoma (Vell.) Arráb. ex Steud., a tree species native to South America, showed higher DQI values when greater irrigation sheets were applied due to the increase in DRM, although no influence of the hydrogel was observed (up to 4.5 g per seedling) (Brucker et al., 2017). The hydrogel did not affect the DQI of the C. pyriformis seedlings, which diverges with what was found by Mews et al. (2015) in seedlings of H. ochraceus. The DQI was also superior in the production of E. dunnii seedlings with 3.3 g per liter of hydrogel (Navroski et al., 2015).

Table 3. Mean values (± standard deviation) for dry root mass (DRM), dry aerial mass (DMA), and Dickson’s quality index (DQI) of C. pyriformis seedlings, according to different water regimes after 20 weeks from the transplant.

| Water regime | DRM (g seedling⁻¹) (A) | DMA (g seedling⁻¹) (B) | DQI (B) |
|--------------|-----------------------|-----------------------|--------|
| No irrigation| 2.85±0.40 b           | 4.7±0.29 b            | 2.26±0.21 b |
| 60% FC       | 5.26±0.93 a           | 17.5±4.20 a           | 6.32±1.25 a |
| 100% FC      | 5.73±0.87 a           | 17.6±3.74 a           | 5.2±0.82 a |
| CV (%)       | 33.5                  | 55.9                  | 45.7   |

Different letters indicate significant differences according to (A) Tukey or (B) Kruskal-Wallis’s test (p<0.05). FC= field capacity of soil.

3.3. Survival

Seedling survival was significantly influenced by the hydrogel doses in the treatment without irrigation, showing an increasing linear trend (R²= 0.72). The absence of the hydrogel induced the survival reduction to 24%, though it increased to 65% with the maximum dose (Figure 3), with no negative effect on growth, biomass accumulation, or seedling quality, as previously mentioned. This result can be attributed to the increase in the soil water retention capacity and its higher availability for seedlings with the presence of the hydrogel, given an intense and prolonged water deficit over time, without being phytotoxic with the maximum dose of 9 g per seedling. Navroski et al. (2015) pointed out that the increase in plant height and stem diameter during the production of E. dunnii seedlings in the nursery was related to the improvement of the substrate physical characteristics (i.e. porosity, water retention capacity), associated to the hydrogel application.

For six native savanna species in Brazil (Cerrado), the use of 1,000 mL of hydrogel solution (5 dry g per seedling) during planting in the dry season reduced the mortality of individuals from 41% to 26%, but if planting occurs in the rainy season, its use is unnecessary (Fonseca et al., 2017). After the application of the same dose of hydrogel in the middle of the driest season in P. patula, the reduction in mortality dropped from 31% to 8%, whereas at the beginning or the end, it was not significant (Mudhanganyi et al., 2018). Previous studies corroborate the fact that seedling survival is favored by the hydrogel when there is an intense and prolonged water deficit (Crous, 2017). Otherwise, as demonstrated for 30 tree species belonging to several successional stages, after a year of establishment in the field without water deficit, neither seedling growth nor survival were affected by the application of hydrogel (Barbosa et al., 2013).

The additional cost of using 9 g per seedling of hydrogel in C. pyriformis would be approximately COP $360 (or USD 0.10, exchange rate in january 2021) per seedling. Further in depth financial analyses should be carried out in order to establish the ideal dose from an economic point of view. Moreover, as the abacu is considered to be a climax or late secondary species, factors such as tolerance to shading and drought should be explored in the future to provide an insight into the physiological and morphological adaptation mechanisms involved.

3.4. Seedling wilting stages

All visual categories of wilting stages were identified in the treatment in the absence of irrigation during the 20 weeks of evaluation (see Figures 4 and 5). Leave angle variation from -45° to -90° (with respect to the horizontal plane) was a key aspect to distinguish category 2 (wilted) and...
Effects of Hydrogel Use in *Cariniana pyriformis*...

3 (severely wilted) (Figure 4C and 5D). Tyree et al. (2003) and Engelbrecht & Kursar (2003) evaluated desiccation tolerance and its relationship with survival in the field as well as in a nursery for 28 tropical species in Panama, using the visual wilting stages applied in the current study, thus confirming its robustness.

Figure 4. Symptoms of wilting stages of *C. pyriformis* seedlings classified in six categories. 0 = normal (A); 1 = slightly wilted (B); 2 = wilted (C); 3 = severely wilted (D); 4 = nearly dead (E) and 5 = dead (F). scale bar= 10 cm. Adapted from Engelbrecht & Kursar (2003).

Seedlings showed no significant differences in category 0 (normal) according to the hydrogel doses used (duration of 39 to 47 days). The dose of 6 g per seedling kept the seedlings in category 1 (slightly wilted) for a more extended period, i.e., 29 days, while with the other doses, the period lasted 20 days (Table 4). Higher doses of the hydrogel induced the later onset of category 3 (wilted), that is, when the first symptoms of leaf necrosis appear. The duration of the categories 0 to 2 had the following relationship concerning the hydrogel doses: 0 g < 6 g < 3 g < 9 g per seedling, that is, 93, 102, 100, and 109 days. The effect of water restriction could gradually reduce cell growth and expansion, and the loss of turgidity and flexibility of the cell wall in those first withering categories led to a scarce seedling support capacity (Le Gall et al., 2015).

Once the seedlings reached categories 3 (severely wilted) and 4 (nearly dead), the tendency towards mortality was almost irreversible, and therefore, there were no significant differences. At the end, category 5 (dead) was shorter with the dose of 9 g (4 days) followed by 6 g (9 days), although the latter showed no differences compared with the other hydrogel doses.
Table 4. Mean values (± standard deviation) for the duration, in days, of the visual wilting stages of *C. pyriformis* seedlings classified in six categories, from transplant up to 20 weeks.

| Hydrogel (g seedling⁻¹) | 0 **ns** | 1 | 2 **ns** | 3 **ns** | 4 **ns** | 5 |
|-------------------------|----------|---|----------|----------|----------|---|
| 0                       | 39.1±1.4 | 16.2±6.8 b | 37.7±3.7 | 16.3±1.6 | 12.1±2.1 | 14.0±3.2 a |
| 3                       | 46.1±2.9 | 21.5±2.3 b | 34.9±5.9 | 14.0±2.4 | 10.3±2.9 | 10.7±3 a |
| 6                       | 41.1±4.5 | 28.9±1.8 a | 28.3±6.8 | 16.3±1.6 | 13.1±3.5 | 9.3±3.6 ab |
| 9                       | 47.6±0.8 | 23.4±4.0 b | 38.7±5.3 | 12.6±2.8 | 12.1±3.5 | 3.7±3.3 b |
| CV (%)                  | 7.8      | 16.6 | 15.1     | 12.6     | 12.0     | 39.3 |

Different letters in the column indicate significant differences according to Tukey’s test (*p* < 0.05). **ns** = not significant. category 0 = normal; 1 = slightly wilted; 2 = wilted; 3 = severely wilted; 4 = nearly dead; 5 = dead. Adapted from Engelbrecht & Kursar (2003).

Figure 5. Symptoms of wilting stages of *C. pyriformis* seedlings classified in six categories, adapted from Engelbrecht & Kursar (2003). 0 = normal (A); 1 = slightly wilted (B); 2 = wilted (C); 3 = severely wilted (D); 4 = nearly dead (E) and 5 = dead (F). Scale bar= 25 cm.

4. CONCLUSIONS

Under agricultural nursery conditions at 50% shading and using sandy loam soil for *Cariniana pyriformis* seedlings of five months of age, the following conclusions were obtained:

- After five months without irrigation, the application of 9 g per seedling of hydrated hydrogel increased the survival of seedlings from 24 to 65%.
- Despite the increase in growth when 9 g per seedling of the hydrated hydrogel was applied, the benefits were not evident (including the effects on the biomass), when the soil is maintained at 60% or 100% of field capacity.
- The onset of wilting symptoms due to water stress tends to be delayed as the dose of hydrated hydrogel increases to 9 g per seedling.

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