Journal of Tropical Biology and Conservation 16: 213–227, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Research Article

Effects of mechanical and acid scarification on germination performance of *Schizolobium parahyba* (Fabaceae - Caesalpinioideae) seeds

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Abstract

Improving seed germination of native species is fundamental for assisting restoration practices, particular in highly degraded ecosystems such as tropical moist forests. Tropical moist forests of Central and South America continue to decrease as a result of fragmentation and conversion of forested land to agriculture. *Schizolobium parahyba* is a pioneer legume tree species widely used in restoration practices due to its fast growth rate, nitrogen-fixing capacity, and wood properties. Seeds of this species exhibit low germination as a result of physical dormancy, which highly limits its propagation on a large scale. We evaluated the effects of mechanical and acid scarification treatments with solutions of sulfuric (10%, 20%) and chloridric (25%, 50%, 75%) on *Schizolobium parahyba* seed germination. Mechanically scarified seeds had higher germination percentage (92.5%) than seeds treated with chloridric acid (50%), sulfuric acid (33.13±.2.11%) or intact seeds (17.5%). Seeds soaked in 10% sulfuric acid for 1 and 5 minutes exhibited higher germination values than seeds soaked in 20% for 10 minutes. Seeds soaked in 75% and 50% chloridric acid solutions for 5 and 10 minutes had an overall higher and faster germination than seeds soaked in 25% for 1 minute. This study indicates that mechanical scarification and acid scarification with solutions of chloridric acid solutions of 50% and 75% can highly improve large-scale propagation of *S.parahyba* and thus assist habitat restoration and conservation practices in degraded moist tropical forests.

Keywords: endangered ecosystems, physical dormancy, seed conservation, seed germination, seed vigour, tropical trees.

Introduction

Improving seed germination rates of native species is fundamental for assisting restoration practices, particular in highly degraded ecosystems. Tropical forests play important roles in biodiversity conservation, carbon storage and climate

Received 21 January 2019
Reviewed 09 July 2019
Accepted 07 August 2019
Published 15 October 2019

regulation (Spracklen et al., 2015). However, about 1.1 million km^2 of tropical forest has been lost over the period 2000 to 2012 (Hansen et al., 2013). From 2010 to 2015, the natural forest area has decreased by a net 6.5 million ha per year (FAO, 2016). Tropical forest cover in South America continues to decrease at an annual rate of 0.40 million ha (FAO, 2016) as a result of habitat fragmentation, logging, fire, and conversion to agriculture and other land uses (Celis & Jose 2011; Gustafsson et al., 2016). Ongoing tropical deforestation threatens global biodiversity and ecosystem services significantly (Spracklen et al., 2015) and global climate change is largely altering species distribution, composition and forest structure (Deb et al., 2018). Therefore, restoration efforts are highly needed to recover at least some of the functions and diversity of heavily degraded tropical forests (Chadzon, 2003).

Forest restoration practices of degraded habitats often involve the establishment of single-species tree plantations that increase site fertility and thus facilitate native forest succession (Chadzon, 2003). Pioneer plant species are particularly important because they can ameliorate above- and below-ground environmental conditions and thus facilitate the establishment of late-successional native species (Rodrigues & Rodrigues, 2014). Rapidly-growing tree species, particularly nitrogen-fixing legumes, can increase organic matter in the soil, prevent erosion, and enhance nutrient cycling (Chadzon, 2003). Direct seeding, a technique in which seeds are introduced directly on regeneration sites, has been shown to improve disturbed habitats in the tropics (Bonilla-Mohello & Holl, 2010; De Souza & Scariot, 2014; Hossain et al., 2014; Muñoz-Rojas et al., 2016). However, before starting a forest restoration programme with native tree species, it is essential to know the germination requirements of the species to be used (Ferreira & Santos, 2012). The knowledge of seed germination requirements is thus fundamental to assist habitat restoration practices and the reestablishment of native plants (Blakesley et al., 2002).

Schizolobium parahyba (Vell. Conc.) S. F. Blake (Fabaceae–Caesalpinioideae) is a pioneer deciduous tree species found mostly in gaps and along forest borders from southern Mexico to Brazil (Maldonado & Escobar, 1999). Mature trees typically have a straight trunk, up to 40 meters tall and 80 cm wide, that branches out only near the top. This tree species is widely used in silvicultural, agroforesty, and restoration practices due to its fast growth rate (it can reach up to 8 -10 m tall after 2 years) and nitrogen-fixing capacity (Lorenzi, 2002). The ability of fixing nitrogen allows this species to survive in poor-quality soils and hence facilitates the establishment of native species by improving soil fertility (Orwa et al., 2009). Trees also provide shade in coffee plantations and

are tolerant to a wide range of rainfall levels, temperatures and soil conditions (http://www.worldagroforestry.org). Its lightweight wood (density 0.32 g/ cm^3) is used to make boxes, liners, boards, matches, toys, model aircrafts (Freire et al., 2015) light construction, furniture, and handicrafts (Oliveira Silva et al., 2018). Trees are also highly valued as ornamentals for roadsides and urban planting.

Despite its ecological importance, low and erratic germination due to physical seed dormancy highly limits its large-scale propagation (Carvalho, 2005). Physical dormancy is caused by one or more water-impermeable layers of palisade cells in the seed or fruit coat (Baskin & Baskin, 2014). Physical seed dormancy can be overcome with mechanical and chemical scarification methods that disrupt the seed coat allowing water movement to the embryo (Baskin & Baskin, 2014). Pre-sowing treatments such as cold stratification, mechanical disruption, or immersion in acid and hot water are widely used because they can improve germination within a relatively short period (Tadros et al., 2012). Although several chemical and mechanical methods have been used to break the physical dormancy of *S. parahyba* seeds (Alves de Azeredo et al., 2003; Freire et al., 2007; Yubero, 2011; Castro Nina, 2016), large-scale propagation programmes and restoration practices of degraded habitats still require rapid and uniform seed germination of *S.parahyba* (Mateus Alves et al., 2017). Testing scarification treatments is thus necessary to further improve seed germination rates of *S.parahyba*. In this study we evaluated the effects of several pre-sowing treatments such as mechanical scarification (sandpaper) and acid scarification with solutions of sulfuric acid (10% and 20%) and chloridric acid (25,50 and 75%) for 1,5 and 10 minutes on the germination of *S. parahyba* seeds. The effects of these acid concentrations and times of immersion have not been tested previously in the seeds of this species.

Materials and Methods

Mature seeds were randomly collected from about 50 trees located in a five ha forest plantation located 35 km south of the town of San Luis, Antioquia, Colombia (05° 37' 54''N, 75°02'02''W, 450 m.a.s.l). This forest plantation was established using seeds from outstanding phenotypes of different forest populations in Colombia. Approximately 100 seeds were collected from each tree. Seeds were collected from selected trees that exhibited straight trunks, high quality wood, high seed production, and represent about 70% of the population of the best trees in the plantation (Rodriguez and Nieto, 1999).

Brown, flat ovate freshly-harvested seeds were about 25 mm in length, had a moisture content of 8% (fresh weight), with approximately 2,200 seeds per kilogramme. In the laboratory, seeds were cleaned with Tween® 20 for 1 minute and rinsed with distilled and deionised water. After cleaning, air-dried seeds were stored in paper envelopes under ambient laboratory conditions (21-23°C, 50% RH) for approximately 90 days until the beginning of the germination trials. Imbibitions curves for intact and mechanically scarified seeds were determined. Two samples of 50 seeds at each condition were tested. Initial weight and fresh weight of each seed sample were measured each hour with an analytic balance until the seed weight had stabilised. Seed viability was determined using 1% tetrazolium solution (2, 3, 5-triphenyl tetrazolium chloride) following ISTA (2012). Two samples of 50 seeds were used. Seeds were soaked in the Tetrazolium solution in flasks totally wrapped with aluminium foil, which were placed in an oven at 37ºC for 22 h. We previously determined that embryo staining was completed within 22h. After this period of time, embryo coloration patterns were evaluated under a dissecting scope. Seeds were recorded as viable when embryos where homogeneously stained (i.e., both radicle and cotyledons).

Germination trials

We conducted germination trials to test the effects of mechanical and acid scarification on *S. parahyba* seeds using a factorial experimental design. Seeds were mechanically scarified by laterally rubbing them against sand paper sheets to make a small hole in the seed coat. Seeds were immersed in solutions of sulfuric acid at 10 and 20% and in solutions of chloridric acid at 25%, 50% and 75% for 1, 5 and 10 minutes to test the effect of acid scarification on germination. Seeds were then placed inside 400 ml flasks. Flasks were placed on a shaker plate so seeds were equally exposed to the acids. After being immersed seeds were then rinsed with distilled and deionised water for 10 minutes. Eight replicates of 10 seeds were used in each of the 16 treatments (including the control treatment) for a total of 1,280 seeds. Seeds were sown in 90 mm-diameter glass Petri dishes on filter paper wetted with 10 ml distilled, deionised water. The dishes were placed in a growth chamber at 24°C, 50% RH and 12-/12-h photoperiod. Germination (radicle protusion was recorded two to three times per week up to 20 d (when no more germination was observed). Seeds were watered as needed.

Data analysis

For each replicate, final germination percentage (GC) and the following germination rate indices were calculated: Germination Rate Index (GRI) = G1 / T1 + G2/T2 + G3/T3 +...Gn/Tn, where, G1=number of germinated seeds on T1;

T1=days of first counting and Gn=number of germinated seeds between Tn-1 and Tn; Tn=days at the final counting; time (d) to reach 50% of maximum recorded germination (R50'); peak value (PV), a measure of seed vigor, calculated as the maximum cumulative percentage germination on any day divided by the number of days to reach that percentage (Czabator, 1962); mean daily germination (MDG), the accumulated percentage of germinated seeds at the end of the test period; and germination value (GV), which expresses the germinative energy and the speed of germination and calculated as GV = MDG x PV.

Differences in each of the germination indices (GC, GRI, PV, R50', MDG and GV) among all treatments were examined by 1-way ANOVA. Tukey's HSD post-hoc tests for multiple comparisons of means were used. Two-way ANOVA tests were conducted to examine the proportion of variation explained by acid concentration and time of seed immersion as fixed factors. Finally, a principal components analysis (PCA) was performed on all multivariate trait data (germination indices) to examine patterns of co-varying traits. Data normality and homogeneity of variances were examined with Kolmogorov-Smirnov tests and Levene's tests (Sokal & Rohlf, 1995). All analyses were performed in JMP 14 (SAS Institute, Cary, NC, USA).

Results

Patterns of seed water uptake differed significantly between intact and mechanically scarified seeds (t = 4.79, P <0.0001). While seed water uptake of intact seeds was 0%, seed water uptake of mechanically scarified seeds was 34.09%. Water uptake of scarified seeds was slow during the first 4 h but increased fast over the next 6-13 h of imbibition, and reached a plateau after 14-16 h. Based on tetrazolium testing, the viability (homogeneously stained embryos) of *S. parahybum* seeds was 90%.

Table 1. Treatment effects of acid scarification (concentration, time and its interaction) on *S. parahybum* percentage seed germination (GC), germination rate index (GRI), time to 50% of maximum recorded germination (R50'), peak value (PV), mean daily germination (MDG), and germination value (GV). Values are percentage of total variation (%) explained by each effect (Type I sum of squares for each effect as percentage of total sum of squares).

Acid	Source of variation	GC	GRI	PV	R50	MDG	GV
Sulfuric	Concentration (C)	25.69*	16.94*	2.04	17.31	25.69*	2.35
	Time (T)	73.01**	78.35**	68.34**	35.60	72.01**	81.26**
	C x T	2.30	4.71	29.61*	44.55	2.30	16.40
Chloridric	Concentration (C)	73.78*	67.79**	30.51*	27.08	73.78*	53.76*
	Time (T)	20.92	23.98*	3.46	12.60	20.92	6.33
	C x T	5.31	8.24	66.03*	60.32	5.31	39.91

*,**Significant at $p \leq 0.01$ and $p \leq 0.001$, respectively.

Germination percentage, rate and vigour differed significantly among seeds subjected to different treatments according to the germination indices ($F_{GC,}$ = 16.40; P <0.0001; F_{GRI} = 20.86; P<0.0001; F_{PV} =10.19, P <0.0001; F_{MDG} =16.40, P <0.0001; F_{GV} =12.58, P <0.0001, Figure 1). Overall, mechanically scarified seeds had on average germination index values (except for R50') significantly higher than seeds soaked in chloridric, sulfuric acid or intact seeds, respectively (Figure 1). Mechanically scarified seeds had on average significantly higher final germination percentages (92.5±2.5%) than seeds soaked in chloridric acid (50.03±2.09%), in sulfuric acid (33.13±.2.11%) or intact seeds (17.5±1.64%).

Acid concentration and time of immersion significantly affected seed germination (Table 1). However, the magnitude of the effect of concentration and time on seed germination differed between sulfuric and chloridric treatments. For seeds soaked in chloridric acid, concentration had a larger effect on germination than time. In contrast, for seeds soaked in sulfuric acid, time of immersion had a larger effect on germination than concentration (Table 1). For both acids, concentration and time interacted significantly only for PV (Table 1). Overall, seeds soaked in 10% sulfuric acid for 1 and 5 minutes exhibited higher germination values than seeds soaked in 20% sulfuric acid for 10 minutes (Figure 1). Seeds soaked in 75% and 50% chloridric acid solutions for 5 and 10 minutes had higher mean germination than seeds soaked in 25% chloridric acid solution for 1 minute (Figure 1). The first two axes of the PCA explained 72.9% and 20% of the variation in the six germination indices (Figure 2). Mechanically scarified seeds and seeds treated with 75% chloridric acid exhibited the highest values for most germination indices (except for R50'). In contrast, control seeds and seeds treated with 20% sulfuric acid exhibited the lowest values. Seeds treated with 10% sulphuric acid and with 25%, and 50% exhibited intermediate values (Figure 2).

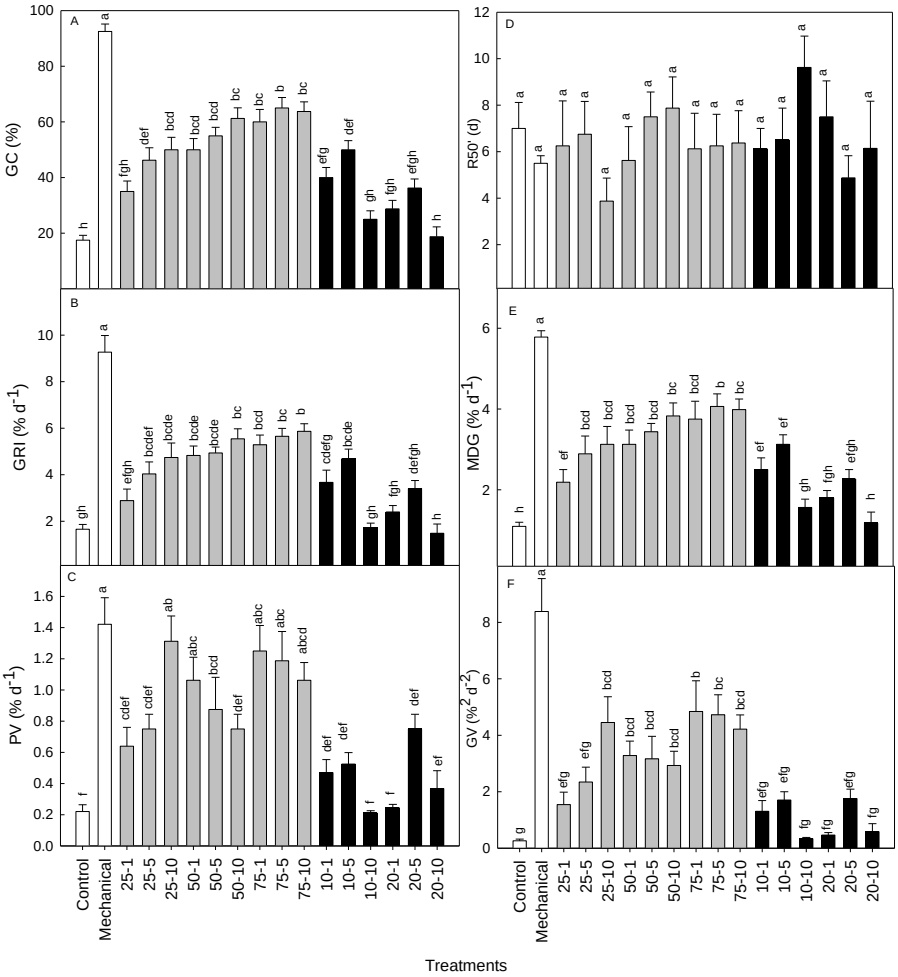

Figure 1. Percentage germination (GC) and germination indices of *Schizolobium parahyba* seeds following either mechanical or acid scarification with chloridric acid (gray bars) or sulfuric (black bars) compared to intact (control) seeds. GC: percentage germination; PV: peak value; GRI: germination rate index; MDG: mean daily germination; R50': time to 50% of maximum germination; GV: germination value. Bars are means ± SE (n=8). Bars topped with different letters differ significantly ($P < 0.05$) according to Tukey's HSD post-hoc tests.

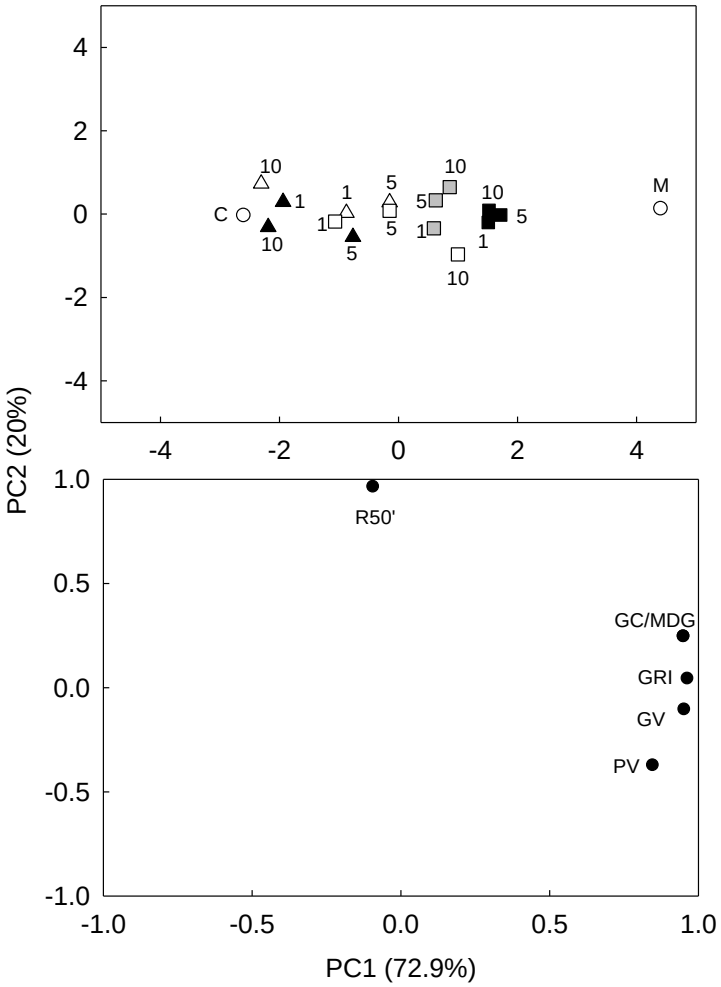

Figure 2. (a) PCA ordination for *S. parahybum* intact seeds (C) and seeds subjected to mechanical (M) or acid scarification treatments with sulfuric acid at 10% (white triangles), 20% (black triangles) and chloridric acid at 25% (white squares), 50% (gray squares) and 75% (black squares) for 1,5, and 10 minutes. (b) Position of germination indices on the first two axes of the principal components analysis for all the treatments included in this study.

Discussion

Mechanical scarification with sand paper and soaking seeds in acid solutions improved germination of *S.parahyba* seeds. Mechanically scarified seeds, however, had higher germination than acid scarified seeds. Our results agree with those of Azeredo et al., (2003), Yubero (2011), Castro Nina (2016) who found that *S. Parahyba* scarified seeds with sandpaper reached 95%, 93% and 97% of final germination, respectively. Oliveira Silva et al., (2018) also found that mechanical scarification and seed immersion in hot water enhance germination rates in the studied species. Mechanical scarification enhances germination in several tropical hard-seeded species because cracks or cuts facilitate water entry and gas exchange which promotes seed germination by activating enzymatic hydrolysis (Missanjo et al., 2013). We observed that coats of freshly harvested seeds of *S. parahyba* were impermeable to water as intact seeds did not imbibe water but mechanically scarified seeds did. In addition, we observed that embryos were fully developed according to the tetrazolium viability tests. Thus, we confirmed that covering layers restrain water uptake by the embryo in this tree species. In seeds with physical dormancy, prevention of water uptake causes the seed to remain dormant until some factor (s) render the covering layer (s) permeable to water (De Souza et al., 2012). In nature, these factors include high temperatures, widely fluctuating temperatures, fire, drying, freezing/thawing and passage through the digestive tracts of animals (Baskin et al., 2000). The use of scarification methods is well known in overcoming dormancy of seeds with hard impermeable coats as it disrupts seed coats and allows water uptake and further radicle emergence (Baskin & Baskin, 2004). Acid scarification methods are often used to overcome physical seed dormancy (Baskin & Baskin, 2014). For example, sulfuric acid was effective to overcome dormancy in several Fabaceae species such as *Schizolobium amazonicum* seeds (Cruz et al., 2007), *Senna obtusifolia*, *Crotalaria senegalensis* and *Crotalaria verrucosa* (Okonwu & Eboh, 2017). However, the efficiency of the treatment varies with the concentration of the acid, plant species and treatment duration (Cruz et al., 2007; Okonwu & Eboh, 2017).

In our study, the type of acid, concentration and time of seed exposure affected germination. Seeds treated with 10% sulfuric for 1 and 5 minutes acid had higher germination than seeds treated with 20% for 10 minutes. Thus, exposure of seeds to sulfuric acid solutions of concentrations higher than 10% for more than 5 minutes may have affected negatively seed coat integrity and/or the plugged natural openings in the studied species. Our results agree with those of Oliveira Silva et al., (2018) who found that *S. parahyba* seeds immersed in concentrated sulfuric acid for 10 and 20 min had lower seed vigour than mechanically scarified

seeds or seeds immersed in hot water. Oliveira Pereira et al., (2011) and Mateus Alves et al., (2017) also found that mechanical scarification was a more effective method to overcome seed dormancy than acid scarification.

In our study, seeds treated with 50-75% chloridric acid for 5-10 minutes exhibited higher germination than seeds treated with 25% for 1 minute. Our results are consistent with Okonwu & Eboh, 2017 who found that chloridric acid at 75% and 50% enhanced seed germination of *Crotalaria verrucosa* (Okonwu & Eboh, 2017) and *Parkia biglobosa* (Abubakar et al., 2013), respectively. Further studies are necessary to test other concentrations of chloridric acid along with different soaking times in the seed germination of *S. parahyba*. In addition, studies have found that seed origin may affect seed germination and seedling vigour in *S. parahyba* (Canchignia-Martínez, 2007; Freire et al., 2015). Thus, further studies testing the effect of seed origin on the germination of this species are necessary.

Our results indicate that mechanical scarification and soaking in chloridric acid at 75% or 50% for 10 minutes enhance seed germination of *S. parahyba*. Therefore these pre-germinative treatments can be used to increase and synchronize germination particularly during nursery stages of restoration projects (Basto & Ramírez, 2015) or to support direct field seed sowing and seedling planting in habitat restoration practices (Heeleman et al., 2012; Muñoz-rojas et al., 2016). In natural conditions, exposure to high constant temperatures or fluctuating temperatures is the most likely cause of release from physical dormancy of *S. parahyba* seeds (De Souza et al., 2012). Seed responses to temperature changes in the habitat usually increase seed coat permeability to water. Studies with species from tropical forests have shown that alternating temperatures on a wet surface effectively broke physical dormancy of seeds (De Paula et al., 2012). Therefore, restoration projects involving seedling establishment of *S. parahyba* in tropical degraded-habitats would highly benefit if seeds are previously scarified and sown during the rainy season to increase the likelihood of seedling survival and growth because the transition from germinated seed to emerged seedling has been identified as the life-stage transition most limiting the success of direct seeding (Muñoz-Rojas et al., 2016). Direct seeding studies indicate that *S. parahyba* is a successful species for restoring tropical moist forests (Engel & Parrotta 2001). In abandoned agricultural lands in Southeastern Brazil, adequate seed germination and survival as well as rapid growth during the first two years after sowing, yielded developing *S. parahyba* forests that facilitated natural regeneration by native woody shrub species in their understories (Engel & Parrotta 2001). Further studies however, are necessary to continue improving restoration techniques,

select appropriate microsites for direct seeding and seedling planting to increase population sizes of *S.parahyba* in the field (Hüller et al., 2017).

Conclusion

Our study shows that mechanical scarification and seed immersion in acid solutions improve germination of *Schizolobium parahyba* seeds. Mechanically scarified seeds had higher germination percentage (92.5%) than seeds treated with chloridric acid (50%), sulfuric acid (33.13±.2.11%) or intact seeds (17.5%). Acid concentration and time of immersion significantly affected seed germination but the magnitudes of the effects of concentration and time on seed germination differed between sulfuric and chloridric treatments. Seeds soaked in 10% sulfuric acid for 1 and 5 minutes exhibited higher germination values than seeds soaked in 20% for 10 minutes. Seeds soaked in 75% and 50% chloridric acid solutions for 5 and 10 minutes had an overall higher and faster germination than seeds soaked in 25% for 1 minute. Therefore, mechanical scarification and seed immersion in chloridric acid solutions of 50% and 75% for 5 and 10 minutes could facilitate large-scale propagation of this pioneer species and thus assist habitat restoration and conservation practices in highly degraded ecosystems such as tropical moist forests.

Acknowledgments

This work was supported by funds from the Pontificia Universidad Javeriana (PUJ) Biology Department. We thank The Biología de Plantas y Sistemas Productivos group at PUJ for logistical support. We are grateful to Corporación Nacional de Investigación y Fomento Forestal (CONIF) for providing seed material. We also thank S. Carrizosa and an anonymous reviewer for providing valuable comments on previous versions of this manuscript.

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