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Enhancing the seed germination process of Montezuma cypress (*Taxodium mucronatum* Ten.)

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Abstract

Montezuma cypress (*Taxodium. mucronatum)* is an ecological, cultural and economically valuable riparian tree species. Two experiments evaluating the effectiveness of various seed treatments were conducted to identify germination best practices and to evaluate the dynamics of the germination process. Seeds were collected on two occasions, one year apart, from the only remaining natural *T. mucronatum* tree stand in the United States. The seeds were subjected to various soaking and stratification conditions. Across all treatments, germinability ranged between approximately 30%-40%, with slightly higher values occurring among the second seed cohort. Overall, no significant differences in germinability were detected in either study, however, soaking seeds in water for 96 hours and stratifying them in moist conditions for 3 weeks significantly accelerated the germination process. Seeds soaked briefly in an NaOH solution followed by a 48-hour water soak demonstrated more synchronous germination than other treatments. Control conditions in which seeds were not soaked or stratified exhibited the slowest germination. These findings are consistent with previous evidence showing that *T. mucronatum* seeds do not exhibit physiological dormancy and that treatments promoting seed water imbibition enhance the germination process. This study adds to the limited available research on *T. mucronatum* propagation practices and offers novel data on the germination parameters of seeds sourced from a natural U.S. stand, rather than seeds from few scattered individual trees, as in previous reports. Seed germination recommendations garnered from this study can improve nursery production of *T. mucronatum* to enhance ecological restoration efforts and ornamental production.

Keywords: seed treatments, plant propagation, germination synchrony, riparian restoration, Rio Grande

Introduction

Cypress trees from the *Taxodium* genus are flood-tolerant species (Duryea et al. 1997) known to provide the wetlands of North America with valuable ecosystem services (Conner et al. 2012). They serve as wildlife habitat, improve water quality, and mitigate hydric erosion (Parresol 2002). The Montezuma cypress (*Taxodium mucronatum* Ten. 1853; syn. *Taxodium distichum* var. *mexicanum*) is the southernmost species of this genus, and has notable cultural and economic significance. It is the official national tree of Mexico, has been regarded by Mesoamerican civilizations as sacred (Sullivan 1994), and is a valuable ornamental tree (Denny and Arnold 2007). Its native range stretches from Guatemala, through many scattered regions of Mexico, up to the southernmost tip of the United States. Although the IUCN lists *T. mucronatum* as a species of least concern globally (Farjon, 2013), its historic prominence in the United States, mostly along the Rio Grande River in Texas, has diminished drastically (St. Hilaire 2001). Aside from planted or isolated individuals in southern New Mexico (St. Hilaire 2001) and the Rio Grande Valley of South Texas, the only remaining natural stand in the United States (2020 emails from A. McDonald and R. Flores; unreferenced) has 69 adult trees (survey by the City of Brownsville in 2014; unreferenced). The population is located in a small section of a former distributary channel (hydrologic feature locally known as a resaca) of the Rio Grande River in Brownsville, Texas. Efforts to restore *T. mucronatum* populations in the United States and to increase its commercial availability as an attractive ornamental tree require effective seed germination strategies. Vegetative cutting propagation has been reported for *T. mucronatum* (St. Hilaire 2003), but the U.S. Fish and Wildlife Service requires that restorative propagation be performed with seeds to promote genetic diversity. Knowledge of *T. mucronatum* seed germination best practices is lacking (Denny and Arnold 2007; St. Hilarie 2001). The scarce available information on seed treatments such as soaking, stratification, and mechanical or chemical scarification indicate very limited success, with germinability percentages remaining low. Denny and Arnold (2007) found that cold seed stratification in moist peat moss, citric acid soaking, and warm water soaking each accelerate germination but do not significantly improve germinability of *T. mucronatum* seeds. Meanwhile, seed coat scarification (knicking seeds) has been found to improve the species' overall germinability (St. Hilaire 2001), but not beyond 20%. Enríquez-Peña et al. (2004) determined that germinability was not influenced by anthropogenic site disturbance and found that temperature minimally impacts the process.

The present study expands on this body of research by evaluating the effects of various seed treatments on the germination process of *T. mucronatum* seeds, as assessed by germinability and other relevant metrics. It consists of two experiments conducted approximately one year apart on freshly collected seeds. Seed treatments evaluated in our study were intended to enhance the germination process, resulting in higher germinability as well as faster and more uniform germination. In Experiment 1 (2018), the ability of seed soaking methods to initiate the germination process by promoting adequate seed water imbibition was assessed. In Experiment 2 (2019), seed stratification treatments were compared to establish the best practice to break seed physiological dormancy, if present. These treatments were hypothesized to ameliorate the process by hastening germination, increasing total germinability, and/or improving synchrony. Five stratification and four soaking treatments (including controls) were tested to identify the seed pre-germination regime(s) that most enhance the germination process.

Materials and Methods

Taxodium mucronatum seeds were obtained from the La Posada del Rey resaca in Brownsville, Texas (25º53'19" N; 97º26'48" W), which is the location of the only natural stand remaining in the United States, population is 69 adult individuals. Mature cones attached to branches or on the ground were collected from multiple trees; seeds were then cleaned, sorted and weighted. For Experiment 1 (2018), 6,593 seeds were obtained in December 2017 and stored for 50 days at 6 ºC prior to application of soaking treatments to a random subsample. The average weight of *T. mucronatum* seeds collected was 7.9 mg ± 0.5 mg (n=100). Seeds used in Experiment 2 (2019) were collected in January 2019 (3,479 seeds) and stored for 35 days at room temperature prior to application of stratification treatments to avoid low temperature dormancy interference.

For both experiments, a completely randomized design was adopted with six replications per treatment. After treatments were applied, seeds were placed on two layers of filter paper moistened with deionized (DI) water and covered by a third filter paper layer in 100 mm petri dishes. Dishes remained covered and paper layers were kept moist at a constant room temperature (23 ºC) under natural photoperiod. Each petri dish (experimental unit) had 50 evenly spaced seeds that were inspected for germination (i.e. protrusion of radicle) every 1-2 days. Germinated seeds were counted and discarded.

Seed treatments compared in Experiment 1 included 48-hour soaking in aerated (using an air stone) DI water, 96-hour soaking in aerated DI water, a 5-minute soak in a solution of 1% NaOH followed by a 48-hour aerated DI water soak, and a control treatment in which no soaking was conducted. In Experiment 2, seed treatments included cold (6 °C) stratification in moist conditions (i.e. between two layers of moistened filter paper in a closed petri dish) for 1 week, moist stratification for 3 weeks, dry stratification for 1 week, dry stratification for 3 weeks, and a non-stratified (23 °C) dry control treatment.

Variables of the germination process measured included germinability (total germination percentage), weighted/mean germination time (average number of days required from maximum germination), mean germination rate (speed of the process, reciprocal of mean germination time), and germination synchrony (degree of spreading of germination over time) (for calculations details see Ranal et al. 2006, 2009). Data was analysed using IBM SPSS Statistics v26. According to Shapiro-Wilks test and Q-Q plots, all data was found normally distributed without transformations; variances were all homogeneous as per Levene's test. One-way analysis of variance was used to assess differences among treatments in both experiments. Means were separated using Tukey's HSD tests ($P<0.05$).

Results

Experiment 1

Overall, germinability for this seed lot was close to 30% regardless of the treatment. Differences among seed soak treatments were not statistically significant but a slightly higher percentage was obtained with the 96-hour soak (Table 1). Statistically significant differences in germination time, rate, and synchrony did occur. The 96-hour water soak treatment resulted in the shortest mean germination time and fastest mean germination rate. The germination process was most synchronous with the 48-hour soak/NaOH bath treatment followed by the 96-hour soak treatment. Seeds in the control treatment (no soak) had the longest germination time as well as the slowest rate and less synchrony. Over the germination period, cumulative germination was consistently highest with the 96-hour soak and lowest for control seeds (Fig. 1).

[Table 1 near here]

Experiment 2

Germinability of this seed lot was higher compared to the lot used in Experiment 1 that was obtained the previous year, reaching around 40% for several treatments. Mean germinability ranged from 30.7% in the dry/1-week stratification treatment to 40.3% in both the control and moist/1-week stratification treatment but these differences were not statistically significant (Table 1). The moist/3-week stratification treatment demonstrated a significantly shorter germination time and faster germination rate than the other treatments but resulted in lower germinability. Both dry stratification treatments resulted in similar, less favourable germination metrics compared to the moist treatments, however germinability appeared higher (not significantly) for the 3-week treatment. The control treatment (no stratification) resulted in the longest, slowest, and most time-dispersed process. Cumulative germination was not consistently distinct for any treatment over the whole period (Fig. 2). Notably, cumulative germination for the moist/3-week treatment was initially highest but ended among the lowest.

[Figure 1 and Figure 2 near here]

Discussion

The highest mean germinability obtained in this study (31.3% in 2018 and 40.3% in 2019) was greater than previously reported for this species (Denny and Arnold 2007; Medina et al. 2005; St. Hilaire 2001), but lower than the 40-50% germinability obtained using chemical treatments on seeds of the closely related bald cypress (*Taxodium distichum*) (Liu et al. 2009). Lower maximum *T. distichum* seed germinability values, such as 24.4% (Popovic et al. 2012) and 26.3% (Krauss et al. 1998), have also been reported. The low germinability characteristic of *T. mucronatum* seeds may be the result of low seed viability (St. Hilarie 2001). Fresh seeds from two locations in Queretaro, Mexico had a viability around 56% based on the tetrazolium test (Enriquez- Peña et al.

2004), suggesting that the germinabilities reported here are relatively high for this species.

The 48-hour soak/NaOH bath treatment appeared to improve the germination process by reducing the mean germination time, accelerating the mean germination rate and resulting in less time-dispersed germination (i.e. higher synchrony). A similar treatment on *T. distichum* seeds resulted in the highest germinability, suggesting that the alkaline solution may facilitate the dissolution of resins on the seed coat and thus improve seed water imbibition (Liu et al. 2009). The 96-hour soak treatment, however, resulted in even more enhanced germination parameters with marked ameliorations on the mean germination time and rate, and a slightly higher germinability. When compared with the control (no soaking), all soaking treatments appear to improve the germination process, suggesting that treatments that increment seed imbibition enhance the germination process, as proposed by Denny and Arnold (2007).

Results from the second experiment suggest that seed stratification generally improves the germination process in this species but not the outcome (i.e. total germinability). Mean germination time, rate, and the synchrony of the process were improved with all stratification treatments compared to the control. Further, the two moist treatments resulted in better germination time and rate compared to the dry stratification treatments. More seed water imbibition likely occurred in the moist stratification treatments, hastening the germination process as soon as the seeds were exposed to favourable temperatures (Denny and Arnold 2007). Moist stratification for 3 weeks accelerated germination. Denny and Arnold (2007) also reported that stratification resulted in earlier germination. However, neither of these studies found that stratification enhances overall germinability, which suggests that *T. mucronatum* seeds do not require a cold period to germinate and thus do not exhibit physiological dormancy. Non-dormant seeds have the capacity to germinate under adequate environmental conditions (temperature, moisture, etc.) (Baskin and Baskin 2004), as was the case in our study. Excised embryos of *T. mucronatum* seeds had no dormancy (St Hilaire 2001). Viability of *T. mucronatum* seeds stored at 2-4 ºC for 21 months was drastically reduced (Enriquez- Peña et al. 2004), again possibly indicating a lack of physiological dormancy.

This study presents information on the dynamics of the germination process of *T. mucronatum* seeds. Derived recommendations should contribute to propagation efforts for both riparian restoration and commercial ornamental production purposes of this symbolic species. Best results were obtained with prolonged seed soaking (using an air stone would be necessary to avoid anaerobic conditions that may result in embryo mortality) and moist stratification (6 ºC, 3 weeks). A combination of these treatments may be redundant as both likely increase seed water imbibition, but further testing is needed. Other seed treatments may influence the germination process as well. Enríquez-Peña et al. (2004) found that the presence of light increased *T. mucronatum* seed germinability. Chemical treatments may also be worth investigating considering their effective application on seeds of the closely related *T. distichum* (Liu et al. 2009) . However, St. Hilaire (2001) reported no germination among *T. mucronatum* seeds treated with sulfuric acid and this study found that soaking seeds in an NaOH solution does not increase germinability but may improve other aspects of the germination process. Considering the inherent low seed viability of this species, other treatments may not result in notable improvements of the germination process compared to what is reported here. This study has additional value because it used a more representative seed sample and source than other reported studies on *T. mucronatum* germination. Previous reports (cited throughout this manuscript) are based on small seed lots obtained from 1-3 isolated individuals, whereas the seeds used in this study were random subsamples from larger lots composed of seeds from multiple trees of a natural stand of 69 adult individuals. Seed viability was high, despite the small number of adult trees. This suggests that restoration of the isolated Montezuma cypress tree population in the Rio Grande Valley of south Texas by seed propagation, promoting genetic diversity, is still possible.

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Declaration of Interest

No potential conflict of interest was reported by the authors

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Table 1. Measurements of the germination process of *Taxodium mucronatum* seeds as affected by various pre-germination treatments. Letters denote significant differences according to Tukey's HSD Test (P<0.05)

Seed Treatment	**Germinability (%)**	**Germination Time (days)**	**Germination Rate (days^{-1})**	**Germination Synchrony**
		Experiment 1		
48 Hour Soak	27.0 ± 2.4 a	7.20 ± 0.42 bc	0.141 ± 0.008 ab	1.06 ± 0.24 b
96 Hour Soak	31.3 ± 1.8 a	4.59 ± 0.13 a	0.219 ± 0.007 c	0.68 ± 0.18 ab
48 Hour Soak + NaOH	28.3 ± 3.1 a	6.18 ± 0.11 b	0.162 ± 0.003 b	0.26 ± 0.17 a
Control (no soak)	27.7 ± 1.5 a	7.68 ± 0.36 c	0.131 ± 0.006 a	1.17 ± 0.11 b
		Experiment 2		
Cold/Moist/1 Week	40.3 ± 2.0 a	6.16 ± 0.44 ab	0.166 ± 0.010 b	2.11 ± 0.09 a
Cold/Moist/3 Weeks	33.7 ± 3.2 a	5.26 ± 0.11 a	0.191 ± 0.004 c	2.09 ± 0.11 a
Cold/Dry/1 Week	30.7 ± 2.8 a	6.67 ± 0.24 bc	0.151 ± 0.006 ab	2.38 ± 0.16 a
Cold/Dry/ 3 Weeks	39.7 ± 2.2 a	6.58 ± 0.14 bc	0.152 ± 0.003 ab	2.02 ± 0.15 a
Control (Warm/dry)	40.3 ± 3.2 a	7.18 ± 0.14 c	0.140 ± 0.003 a	2.45 ± 0.11 a

Figure 1.

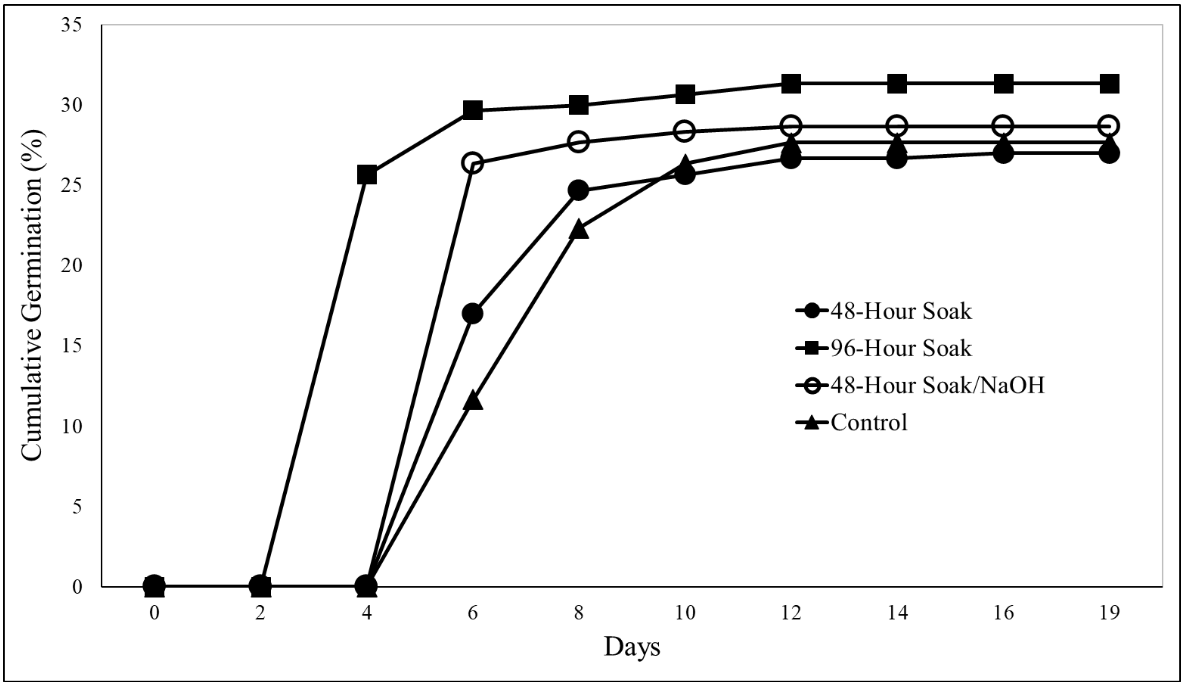

Figure 2.

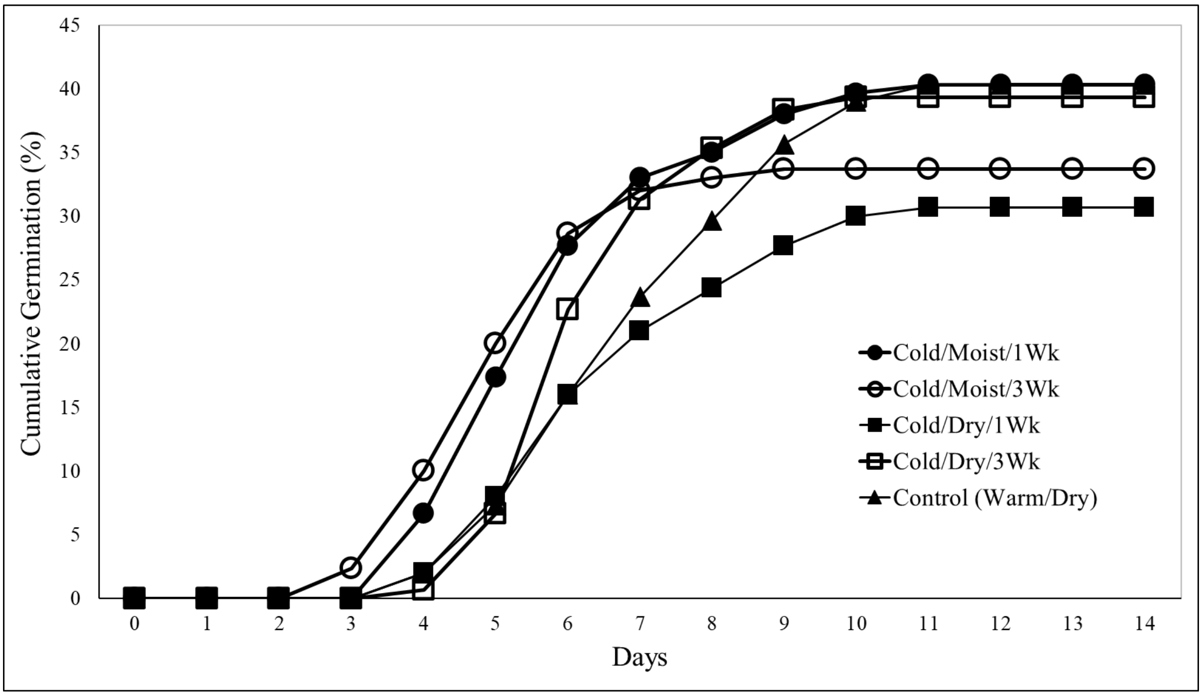

Figure Captions

Figure 1. Cumulative germination of *Taxodium mucronatum* seeds as affected by various soaking treatments.

Figure 2. Cumulative germination of *Taxodium mucronatum* seeds as affected by various cold stratification (6ºC) treatments.