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SHORT COMMUNICATION

Germination rate of endangered cloud forest trees in Mexico: potential for *ex situ* propagation

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ABSTRACT
Deforestation and elimination of reproductive adult trees in Mexican tropical montane cloud forest (TMCF), due to traditional selective logging, threaten the maintenance of more than half of the tree species in this ecosystem. The objective of this study was to evaluate germination in threatened TMCF tree species in order to assess their propagation potential for conservation efforts. Given that seed size is an important trait influencing germination, the relationship between seed size and mean time to germination (MTG) was evaluated. Seeds of 10 valuable TMCF tree species were sown in a controlled environment and germination rates were recorded until reaching their maximum value. All species displayed potential for propagation from seed without pre-germination treatments. Final germination percentages were as follows: *Fraxinus uhdeii* = 36.3 ± 7.4% (mean ± 1 SE), *Juglans pyriformis* = 50.6 ± 6.5%, *Magnolia vovidesii* = 90.8 ± 1.1%, *Meliosma alba* = 68.4 ± 6.8%, *Ocotea disjuncta* = 64.1 ± 12.9%, *Oreomunnea mexicana* = 17.8 ± 4.4%, *Quercus germana* = 18.1 ± 1.9%, *Sideroxylon contrerasii* = 27.1 ± 12.6%, *Ulmus mexicana* = 33.6 ± 3.7%, and *Zanthoxylum melanostictum* = 12.7 ± 2.2%. Time required to reach maximum germination varied widely among species; from 49 days in *F. uhdei* to 434 days in *S. contrerasii*. No significant correlation was found between seed mass and MTG. While *ex situ* germination is an important aspect of propagation to reinforce populations of these species, further assessment of germination and transplanted seedling survival under varied TMCF conditions is required.

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Mean time to germination; restoration; tropical montane cloud forest; seed; tree regeneration

Introduction

Deforestation and degradation of tropical montane cloud forest (TMCF) threaten the high biodiversity that characterizes this ecosystem (Scatena et al. 2010; Toledo-Aceves et al. 2011). While TMCF is considered of low potential for timber harvesting, unplanned selective logging is a common subsistence practice among forest owners (Rüger et al. 2008). As a result of habitat loss and the gradual elimination of reproductive trees, 60% of the TMCF tree species in Mexico have been reported as threatened (González-Espinosa et al. 2011).

Previous studies propose transplantation of native tree seedlings and juveniles as a viable strategy for cloud forest restoration (Ramírez-Marcial 2003; Alvarez-Aquino et al. 2004; Montes-Hernández & López-Barrera 2013). To support reintroduction programs and reinforcement of populations via enrichment plantations, for example, propagation from seeds of native TMCF tree species is required in order to maximize genetic variability. However, the propagation potential from seed of valuable tree species is often unknown (but see Camacho-Cruz et al. 2000; Alvarez-Aquino et al. 2005), particularly for scarce and endemic species, the seeds of which can be difficult to obtain. Germination is one of the most important stages in the life cycle of trees and variation in germination rate may have important implications for success; interspecific variation in germination rates may translate into competitive differences among species during regeneration and imply differential risks of predation (Poorter & Rose 2005; Cao et al. 2011).

Seed size is one of the most important traits influencing germination (Pearson et al. 2002). Seed size represents a fundamental trade-off between producing more small seeds and fewer larger seeds (Leishman et al. 2000). This trait has been related to variation in time to germination (Pearson et al. 2002; Soriano et al. 2011). Since large seeds are more likely to suffer higher post-dispersal seed predation than small seeds, large-seeded species are in theory expected to germinate faster than small-seeded species (Norden et al. 2009). Nevertheless, a meta-analysis incorporating 1037 tree species from five tropical areas shows that large seeds do not germinate faster than small seeds, but rather that small seeds germinate faster (Norden et al. 2009).

In this study, the objective was to evaluate the relationship between seed size and germination rate in valuable TMCF trees under controlled conditions and to assess their propagation potential for management practices. Basic information regarding the germination of TMCF tree species is fundamental to the accurate assessment of their potential for management and conservation of endangered taxa.

Methods

To evaluate germination, seeds of 10 native tree species were collected from TMCF fragments in the subwatershed of the River Pixquiac, in central Veracruz,

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Table 1. Tree species of tropical montane cloud forest for which seed germination was assessed.

Species	Family	Status[1]	Shade tolerance	Fruit type	Seed collection date	Seed size (g)	MTG (d)
Fraxinus uhdei (Wenz.) Lingelsh.	Oleaceae	LC	S[2]	Samara	October 2013	0.02	17.4
Juglans pyriformis Liebm.	Juglandaceae	EN	I[3]	Nut	December 2013	26.51	93.2
Magnolia vovidesii A.Vázquez, Domínguez-Yescas & L. Carvajal	Magnoliaceae	EN	S[4]	Polyfollicle	October 2013	0.26	39.4
Meliosma alba (Schltdl.) Walp.	Sabiaceae	EN	I[5]	Drupe	October 2013	0.09	92.2
Ocotea disjuncta Lorea-Hern.	Lauraceae	EN	S[6]	Drupe	January 2014	2.45	135.2
Oreomunnea mexicana J.F. Leroy	Juglandaceae	EN	S[7]	Samara	January 2014	0.18[7]	77.9
Quercus germana Schltdl. & Cham.	Fagaceae	CR	I[6]	Acorn	November 2013	18.13	90.2
Sideroxylon contrerasii (Lundell) T.D. Penn.	Sapotaceae	VU	NA	Berry	October 2014	4.76	316.6
Ulmus mexicana (Liebm.) Planch.	Ulmaceae	EN	S[2]	Drupe	February 2014	0.002	50.4
Zanthoxylum melanostictum Schltdl. & Cham.	Rutaceae	LC	S[2]	Drupe	February 2014	0.02	106.7

[1] González-Espinosa et al. (2011); [2] Ramírez-Marcial et al. (2012); [3] Pedraza and Williams (2003); [4] Sánchez-Velásquez and Pineda-López (2006); [5] Muñiz-Castro (2008); [6] Muñiz-Castro et al. (2015); [7] Atondo-Bueno et al. (2016).
Conservation status: EN = endangered; VU = vulnerable; NT = near threatened; and LC = least concern. Shade tolerance: S = shade tolerant; I = intermediate NA = not available. MTG = mean time to germination (d = days).

Mexico (Table 1). Species selection was based on the high value ascribed to these species by TMCF landowners, their present low abundance in the region, and the availability of seeds. All species are included in the Red List of Mexican Cloud Forest Trees (González-Espinosa et al. 2011; status shown in (Table 1)). Voucher specimens of all species were deposited at the Herbarium of the Institute of Ecology, A.C. Mexico (IE-XAL). Hereafter, the tree species will be referred to by their genus. The number of trees from which seeds were collected are as follow: *Fraxinus* = 2, *Juglans* = 3, *Magnolia* = 3, *Meliosma* = 3, *Ocotea* = 3, *Oreomunnea* = 3, *Quercus* = 4, *Sideroxylon* = 5, *Ulmus* = 4, and *Zanthoxylum* = 3. The reduced number of trees available for collection was due to the scarcity of certain species. Seeds were collected directly from the trees to reduce risk of contamination by soil pathogens. The weight of 30 seeds per species was recorded in an analytical balance (Ohaus® AV8101). Seeds were sown immediately after collection (fruit collection month/year is shown in (Table 1)). Before sowing, seeds were placed in buckets with water and those that floated were considered nonviable or empty and discarded. Seeds from each species were placed in a minimum of four plastic germination trays (45 cm × 32 cm × 10 cm) with a mixture of black soil and tepezil (3:1). Seed size and availability dictated the number of trays used in each case: *Fraxinus* = 5 trays, *Juglans* = 5, *Magnolia* = 6, *Meliosma* = 5, *Ocotea* = 4, *Oreomunnea* = 8, *Quercus* = 9, *Sideroxylon* = 5, *Ulmus* = 4, *Zanthoxylum* = 4. The total number of seeds sown per species is provided in the legend of Figure 1. Seeds of *Juglans, Magnolia, Meliosma, Ocotea, Quercus, Sideroxylon,* and *Zanthoxylum* were imbibed in water for 24 h prior to sowing. The sarcotesta of *Magnolia* and the mesocarps of *Sideroxylon* seeds were manually removed prior to sowing. No other pre-germinative processing was applied. All seed trays were kept in a greenhouse at the Botanical Garden "Francisco Javier Clavijero" (Veracruz, Mexico; 19° ′ 37″ N 96° 56′ 36″ W; 1250 m asl) and watered every 3 days. Temperature and relative humidity were measured with temperature and relative humidity sensors (onset Hobo® Microstation Data loggers and HOBO 12-Bit Temperature/RH Smart Sensor) placed at plant level over a period of 5 days in July 2015. Mean temperature was: 19.46 ± 0.17°C, and relative humidity 63.86 ± 1.40%. Seeds were considered to have germinated on emergence of the radicle. Germination was recorded every 3 days initially, then weekly and monthly depending on the species, until no further germinated seeds appeared in consecutive recordings. Mean time to germination (MTG) was calculated for each species according to the formula:

$$t = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i},$$

where t_i = time from the start of the experiment to the ith observation (day), n_i = number of seeds germinated at the ith time (the number corresponding to the ith observation), and k = time of the last germination. A Pearson correlation was performed between the log-transformed values of MTG and the log-transformed values of seed mass for the 10 species (Norden et al. 2009) using MINITAB 16.

Results and discussion

All of the species displayed potential for propagation from seed as a viable method for conservation. Final germination values were high for most of the species evaluated: *Fraxinus* = 36.3 ± 7.4% (mean ± 1 SE), *Juglans* = 50.6 ± 6.5%, *Magnolia* = 90.8 ± 1.1%, *Meliosma* = 68.4 ± 6.8%, *Ocotea* = 64.1 ± 12.9%, *Oreomunnea* = 17.8 ± 4.4%, *Quercus* = 18.1 ± 1.9%, *Sideroxylon* = 27.1 ± 12.6%, *Ulmus* = 33.6 ± 3.7%, and *Zanthoxylum* = 12.7 ± 2.2%. *Magnolia* presented the highest germination, with similar values to those reported previously by Corral-Aguirre and Sánchez-Velásquez (2006), while *Oreomunnea* displayed low values in comparison to the 63% germination reported by Atondo-Bueno et al. (2016) under controlled conditions. For the rest of the species studied, no previously published information was found. Time to begin germination varied greatly among species; from 8 days in *Fraxinus* to 122 in *Sideroxylon*. Time to reach maximum germination also presented a wide range; from 49 days in *Fraxinus* to 434 days in *Sideroxylon* (Figure 1). The MTG ranged from 17.4 in *Fraxinus* to 316.6 in *Sideroxylon* (Table 1). For most of the species evaluated, there appears to be no requirement for pre-germinative treatments; however, for *Sideroxylon* and *Juglans* scarification could be applied in order to prompt earlier and more rapid germination.

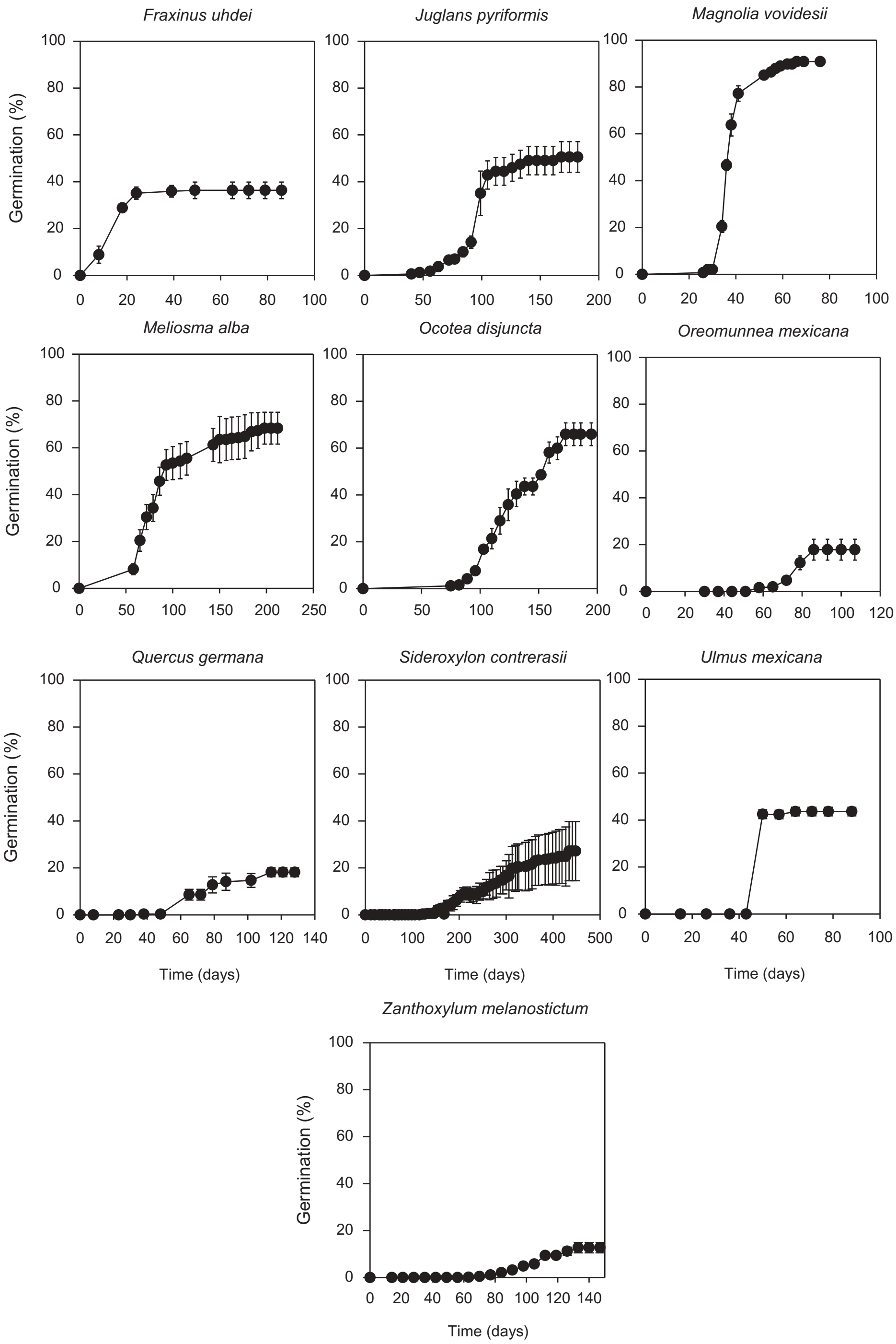

Figure 1. Germination rates (mean ± 1 SE) under controlled conditions of valuable tropical montane cloud forest tree species. Time scale varies dependent on species. Total number of sown seeds per species: *Fraxinus uhdei* = 1500, *Juglans pyriformis* = 170, *Magnolia vovidesii* = 588, *Meliosma alba* = 490, *Ocotea disjuncta* = 260, *Oreomunnea mexicana* = 3200, *Quercus germana* = 600, *Sideroxylon contrerasii* = 312, *Ulmus mexicana* = 800 and *Zanthoxylum melanostictum* = 2250.

Further assays are recommended, including seed viability and sowing under varying conditions of temperature, light, and humidity. For example, temperature fluctuation can stimulate the germination of larger-seeded species (Pearson et al. 2002). Due to variation in fruit production among species, not all germination trials occurred simultaneously and fluctuations in temperature and humidity throughout the study were uncontrolled. An evaluation of seed longevity and different storage conditions would also be valuable in terms of supporting germplasm conservation programs.

Evaluation of germination in TMCF conditions would also provide valuable information for management. Direct seeding of *Oreomunnea* has been reported as a reliable and low-cost technique by which to ensure the regeneration of this species in cloud forest (Atondo-Bueno et al. 2016). In contrast, the low germination rate of *Sideroxylon*, for example, could increase the risk of predation and pathogen attack in the field. A preliminary collection of fruits from the ground revealed the presence of coleopteran larvae within the fruit and this particular set of fruit presented no subsequent germination (unpublished data). While *Sideroxylon* display high seed mass and low MTG overall, the absence of a significant relationship between seed size and MTG (r = 0.54; P = 0.11) could have been influenced by the reduced number of species included in this analysis.

While many cloud forest tree species are currently listed as threatened and have become locally rare, the Mexican Forestry Commission promotes reforestation, with nonnative species, of pasturelands previously occupied by TMCF (Gerez et al. 2012). This study shows that propagation from seeds of valuable species is possible and that the resulting seedlings could be used to reinforce TMCF tree populations. While germination is an important step toward the reinforcement of populations of these species, experiments to assess *ex situ* plant development and establishment in TMCF following transplantation are required in order to support management practices.

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Disclosure statement

No potential conflict of interest was reported by the author.

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