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Direct seeding of *Oreomunnea mexicana*, a threatened tree species from Southeastern Mexico

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Abstract Compared to enrichment planting techniques, direct seeding may represent a viable low-cost method to ensure the conservation and recovery of forest ecosystems. However, it is necessary to identify the environmental factors that affect seed germination and seedling establishment in order to improve the success of this technique. It has been suggested that the establishment of *Oreomunnea mexicana* (Juglandaceae), a threatened tropical montane cloud forest tree species, is associated with microsites of high soil moisture. We assessed seedling emergence in *O. mexicana* through direct seeding in a secondary forest and characterized the microenvironmental conditions of the sowing microsite. We also assessed the effect of seed hydration on seedling emergence and evaluated the effect of soil moisture content and seed hydration in *O. mexicana* seed germination and seedling emergence under laboratory conditions. Seedling emergence was lower in the field than in the laboratory (37 vs. 42 %, respectively). At microsite level, seedling emergence correlated positively with soil moisture content but negatively with vegetation cover. After 11 months, 52 % of the emerged seedlings still survived. Under laboratory conditions, seedling emergence did not differ significantly between hydrated and non-hydrated seeds (43.2 ± 0.52 vs. 40.3 ± 0.51 %, respectively), but did between high and low soil moisture contents (80 ± 0.18 vs. 3.5 ± 0.085 %, respectively). With appropriate soil moisture and vegetation cover conditions, *O. mexicana* seed introduction into secondary forest is a reliable technique. However, the method could be improved by protecting the seedlings from physical damage.

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Introduction

Tropical montane cloud forest (TMCF) is a unique ecosystem that harbors high biodiversity, including a large number of endemic species (Rzedowski 1996; Hamilton et al. 1995; Bubb et al. 2004; Scatena and Bruijnzeel 2011; Williams-Linera et al. 2013). Despite its restricted distribution, TMCF provides important environmental services such as regulation of climate, control of soil erosion, maintenance of water and nutrient cycles and sequestration of carbon (Hamilton et al. 1995; Bruijnzeel 2004; Bubb et al. 2004). Nevertheless, it is one of the most threatened terrestrial ecosystems at the global level (Aldrich et al. 1997; Challenger et al. 2009; Scatena and Bruijnzeel 2011). In Mexico, TMCF is mainly threatened by forest cover change (Muñoz-Villers and López-Blanco 2008), human population growth (Toledo-Aceves et al. 2011) and climate change (Challenger et al. 2009; Rojas-Soto et al. 2012). It was estimated by 2002 that 72 % of the original Mexican TMCF cover had been lost, while 52.4 % of the remaining forest was considered secondary (Challenger et al. 2009). As a result, 60 % of the tree species face some degree of threat (González-Espinosa et al. 2011). In this context, it is important to develop strategies to promote the conservation of cloud forest tree species, as well as the restoration of the TMCF itself.

Currently, the most common strategy to recover tree species and increase woody vegetation is reforestation of abandoned agricultural lands (Lamb et al. 2005). Extensive reforestation is an expensive technique due to the unit cost per tree, seedling transportation, soil preparation, mechanical weed control and/or fertilizer application. In order to reduce these costs, few species with rapid growth are introduced (Summers et al. 2015). The use of rare native tree species in reforestation programs is limited by the lack of ecological knowledge, constant seed supply and technical capacities for propagation of the species. However, there are other less-explored methods, such as enrichment planting of degraded and secondary forests, which can assist the recovery of native and rare forest species (e.g. Ramos and del Amo 1992; Adjers et al. 1995; Montagnini et al. 1997; Peña-Claros et al. 2002; Sovu et al. 2010; Millet et al. 2013). Enrichment planting is particularly important to guarantee the establishment of certain key species that, under natural regeneration, are unable to colonize the site or regenerate by themselves (Lamb et al. 2005). This method represents an alternative for accelerating the recovery of degraded and secondary forests and maintaining valuable species (Montagnini et al. 1997; Lamb et al. 2005; Paquette et al. 2006) and implies the deliberate introduction of seedlings or seeds (i.e. direct seeding) of one or more species of interest.

Direct seeding is a viable, low-cost restoration method (Engel and Parrotta 2001; Cabin et al. 2002; Camargo et al. 2002; Doust et al. 2006; Ceccon et al. 2015). However, in order to assess its feasibility, it is necessary to evaluate the factors that affect seed germination and seedling survival (Doust et al. 2006; Cole et al. 2011). Studies in abandoned grasslands and secondary tropical forests have found that seed germination, as well as seedling emergence and early establishment, are affected by several factors (Holl et al. 2000). These factors include seed and seedling predation (Pedraza-Pérez and Williams-Linera 2005; Montes-Hernández and López-Barrera 2013), light irradiance (Gaviria and Engelbrecht

2015), quantity and type of forest floor litter (López-Barrera and González-Espinosa 2001), presence of exotic grasses (Ortega-Pieck et al. 2011) and soil moisture content (Bentos et al. 2013; Gaviria and Engelbrecht 2015). This latter factor is particularly important, since seed desiccation can act to impede or delay germination (Hegarty 1977). Pre-germination treatments, such as soaking seeds in water (i.e. seed hydration or seed hydropriming; McDonald 2000) may therefore serve to enhance seed germination. Soaking seeds in water sensitizes the seeds, removes chemical inhibitors and softens the seed coat (Ramírez-Marcial et al. 2003; Davies et al. 2011), ultimately facilitating the germination process (Alvarado-López et al. 2014).

Information regarding the environmental requirements for seed germination and seedling emergence is even more important for species that are threatened or those with restricted and discontinuous distribution. This is the case for *Oreomunnea mexicana*, a relict species listed as being under high risk of extinction throughout its restricted distribution in Mexico and Central America (González-Espinosa et al. 2011). Previous studies of this species have described its natural regeneration (Naranjo-Luna 2014; Corrales et al. 2016a), however, for the purposes of conservation and restoration, more detailed information is required regarding assisted regeneration of the species (Avendaño-Yáñez et al. 2014). The objectives of this study were therefore: (a) to assess seedling emergence through direct seeding of *O. mexicana* in an old secondary forest, comparing seed hydration treatments and correlating the results with the local microenvironmental conditions; and (b) to evaluate seed germination and seedling emergence under laboratory conditions, comparing seed hydration and soil moisture treatments. This information will help efforts to develop appropriate management strategies for enriching secondary and degraded forests and thus contribute to the conservation of this species.

Methods

Study species. *Oreomunnea mexicana* (Standl.) J. F. Leroy subsp. *mexicana* belongs to one of the most primitive genera in the family Juglandaceae (Rzedowski and Palacios-Chávez 1977; Blokhina 2007; Herrera et al. 2014). Adult trees reach a height of 40 m and up to 105 cm diameter at breast height. Sexual reproduction of *Oreomunnea* is anemophilous, from unisexual, apetalous flowers with a wind-pollination syndrome. With the asset of the arborescent habit, *Oreomunnea* fruits have probably evolved to adopt the strategy of producing heavy, well-protected, one-seeded, indehiscent fruits. Such fruits are suited to both wind and animal dispersal and, perhaps more importantly, have considerable food reserves that enable a seedling to be independent of photosynthetic activity while establishing vigorous, competitive ability (Stone 1973). *Oreomunnea* fructifies from October to December (Niembro et al. 2010) and seeds are recalcitrant with distinctive trilobed wings for wind dispersion (Stone 1972; Dilcher and Manchester 1986). Seed germination is hypogeal and affected by insect damage, such as that caused by curculio seed-borers (around 15 % of seeds are infested; FAO 2009). *Oreomunnea mexicana* is classified as an endangered species due to its discontinuous and restricted distribution from Mexico to Central America (González-Espinosa et al. 2011). In Mexico, it is located mainly in the central and southern mountainous regions (900–2000 m asl) where precipitation ranges from 1000 to 5000 mm/year (Blokhina 2007; Niembro et al. 2010).

Study site

Fieldwork was carried out in the "Santuario de Bosque de Niebla" (SBN) reserve, located in Xalapa, Veracruz, Mexico (19°30′47.80″N, 96°56′15.93″W; 1250 m asl). The SBN is a fragment of TMCF (30 ha) that presents different successional stages including open areas, secondary vegetation and remnants of old-growth forest. During the first half of the last century, parts of the SBN were occupied by shade coffee plantation, where coffee and citrus replaced the understory vegetation. Since 1975, however, the area has been protected and forest recovery has taken place through elimination of the coffee and citrus and allowing natural succession. The local climate is temperate humid with an average annual temperature of 18.7 °C and mean annual rainfall of 1671 mm, the soil is classified as Andesite (Williams-Linera et al. 2013). The vegetation cover is dominated by *Quercus xalapensis, Q. germana, Carpinus tropicalis, Clethra macrophylla*, *Liquidambar styraciflua* and *Turpinia insignis* (Williams-Linera et al. 2013).

Seed collection

Seeds were collected in January 2014 from two adult individuals of a small population of *O. mexicana* located in Tlalnelhuayocan, Veracruz, Mexico (19°30′0.86″N, 97° 0′31.14"W). Seed collection was restricted to this area since permission to collect seeds from other fruiting individuals in adjacent areas was not granted by the landowners. The vegetation cover in the area corresponds to tropical montane cloud forest fragments of varying conservation status. Seeds were removed from the branches (when the embryo was pink it was considered a mature seed), then cleared by removing the husk and stored in plastic bags at room temperature until subsequent use. The seeds were pre-selected using the flotation method (Gribko and Jones 1995), with floating seeds discarded since they may have been empty or damaged. Using this method, seed viability is found to be around 85 % (Pers. Obs. Israel Gómez, Pronatura Veracruz, A.C.) and is lost in less than 3 months (Niembro et al. 2010).

We selected 1200 seeds for each experiment (field and laboratory). In order to determine the effect of seed hydration treatment on seed germination and seedling emergence, half of the seeds were soaked (i.e. hydrated) in tap water for 72 h at room temperature prior to sowing in both of the experiments. A total of 40 non-hydrated and 40 hydrated undamaged seeds were randomly selected and their individual weight, length and width recorded. Non-hydrated seeds presented an average weight of 0.18 g (±0.005), width of 0.69 mm (±0.007) and length of 0.66 mm (±0.008). Hydrated seeds had a weight of 0.21 g (±0.005), width of 0.69 mm (±0.006) and length of 0.69 mm (±0.008). In both experiments, seeds were sown at a depth of 1 cm. We removed the surface litter layer in order to ensure the correct sowing depth under field conditions. Each germinated seed and emerged seedling was marked with a wire tag placed beside the seed or seedling.

Direct seeding

In February 2014, seeds were sown in 60 microsites in the forest interior every 5–10 m along a 600 m transect. Selection of seeding microsites was based on the presence of medium to high forest vegetation cover and apparent high soil moisture content, both of which are appropriate for the late-successional species. Each microsite consisted of two 4 × 10 cm paired plots, separated by an average of 70 cm in distance, such that both plots

shared the same microenvironmental conditions. Ten hydrated seeds were sown in one plot of each pair and ten non-hydrated seeds were sown in the other, with a separation distance of 2 cm maintained between seeds.

To characterize the environmental conditions of each microsite, several variables were recorded (Table 1). Air temperature was recorded with a pocket weather meter (Kestrel 2500); ground level vegetation cover (measured from approximately 30 cm above the soil) was estimated with a convex densiometer (Forestry Suppliers); leaf litter, herbaceous and bare soil cover and topsoil depth (cm) were estimated within an area of 50 × 50 cm, taking each plot as the center. Percentage light transmittance was recorded with a ceptometer (AccuPAR LP-80; PAR) placed above each plot. In order to compare the photosynthetically active radiation (PAR: wavelength of 400–700 nm) available to the plants in each microsite, we used two sensors: a bar sensor was placed under vegetation cover, while an external sensor was exposed to direct sunlight (open site). These two measurements were used to obtain the proportion of available light per microsite. All variables were recorded on the same day in March 2014 apart from the PAR, which was measured in August of the same year. Furthermore, six soil samples were collected in April 2014 from each microsite at depth 10 cm in order to measure soil moisture content, available water capacity and bulk density. Soil moisture content (g g^{-1}) was calculated by drying a subsample of the cored soil at 105 °C to obtain the oven-dry weight of the soil in the core sample (NOM-021-RECNAT-2000). Available water capacity (g g^{-1}) was determined from the difference between the soil water content at field capacity and that of the permanent wilting percentage. These analyses were conducted using a pressure plate apparatus at 0.33 and 15 bars, respectively. Bulk density (g cm^{-3}) was determined by the core method (Blake and Hartge 1986).

Seedling emergence (i.e. percentage of seeds emerged from sown seeds) and survival (i.e. percentage of seedlings surviving from sown seeds) were recorded every 15 days from February 2014 to January 2015. Seedling emergence was recorded when the emerged plumule reached 0.5 cm from the forest floor. One of the main objectives of this study was to evaluate the feasibility of the direct seeding practice in degraded forests and, in order not to affect the soil humidity environment of each seed, we decided not to manipulate and stress each seed in order to evaluate when germination occurred. Where applicable, and possible, causes of mortality were recorded (e.g. desiccation, physical damage and

Table 1 Environmental characteristics of experimental microsites in a secondary tropical montane cloud forest in central Veracruz, Mexico

Environmental characteristics	Min.–Max.	Mean	±1 SE
Air temperature (°C)	15–28	23	0.41
Vegetation cover (%)	60–93	79	0.95
Leaf litter (%)	10–100	62	2.73
Herbaceous cover (%)	0–75	22	1.78
Bare soil cover (%)	0–80	16	2.50
Topsoil depth (cm)	0.5–10	3	0.32
Light transmittance (%)	0.18–15.33	3	0.42
Soil moisture (%)	32–80	48	1.58
Available water capacity (%)	3–16	8	0.44
Field capacity (%)	25–97	58	2.13
Permanent wilting percentage (%)	22–88	50	1.96
Bulk density (g cm^{-3})	0.2–1.06	0.7	0.02

undetermined). At the end of the experiment, maximum height (cm), basal stem diameter (mm) and number of leaves were recorded for each seedling.

Seed germination and seedling emergence under laboratory conditions

In March 2014, we assessed seed germination and seedling emergence as a function of: (1) pre-germination treatment (hydrated vs. non-hydrated seeds), and (2) soil moisture content (20–30 and 50–60 %, simulating two contrasting moisture contents recorded in the SBN). In the laboratory, mean temperature was 22.9 ± 2.65 °C, humidity was 62.5 ± 4.35 % and the indoor daylight was 38.5 ± 10.19 μmol $m^{-2}s^{-1}$. Plastic containers (14 × 14 × 4 cm) with drainage holes in the bottom were filled with approximately 140 g of substrate from the SBN, previously sterilized at 76.6 °C (Pro-grow, model SST-15). Soil moisture content was calibrated to achieve the two experimental levels: 20–30 % (irrigation of 50–55 ml every 7 days to keep the substrate moist) and 50–60 % (irrigation of 50–55 ml every 3 days). Each of the four factor combinations (each with 10 seeds) was replicated 30 times (a total of 120 containers and 1200 seeds). The plastic containers were randomly placed on the laboratory bench and rotated every 7 days.

Seed germination (i.e. percentage of seeds germinated from the total number of sown seeds) and seedling emergence (i.e. percentage of seedlings emerged from the total number of sown seeds) were recorded every four days for the first months and every 15 days thereafter until the end of the experiment. Seeds were considered to have germinated when the radicle exceeded 3 mm in length. Where possible, causes of mortality were recorded (e.g. desiccation, rot and undetermined). In order to monitor the development of emerged seedlings, they were transplanted into black plastic bags (20 × 5 × 5 cm) once they had exceeded 5 cm in height. The plants were approximately 4 months old when transplanted. The seedlings were placed in a nursery adjacent to the SBN under a mesh shade that allowed 30 % light incidence and watered every third day. In order to obtain a control featuring seedlings that had emerged from direct seeding, the total number of leaves, maximum height (cm) and basal stem diameter (mm) of the seedlings were recorded after a period of 11 months.

Data analysis

To analyze germination rate as a function of the experimental factors, differences between the curves of seedling emergence were tested with the Kaplan–Meier survival analysis (Kaplan and Meier 1958) using the SPSS program (v. 11). Differences in seedling mortality per experimental treatment (pre-germination hydration and soil moisture content) were analyzed using a Mann–Whitney *U* test, since the data did not comply with the assumptions of normal distribution. Spearmańs correlation coefficients were calculated for the microenvironmental conditions. Logistic regression models were used to analyze the final proportion of seed germination and seedling emergence (Binary response: 0 = not germinated, 1 = germinated) (Logit function). To test the accuracy of the model, the value of Wald (χ^2) was calculated. We used the variables with the greatest deviance and highest influence in the beta value, since these variables are the most representative of each model and allowed the model to be chosen based on parsimony. In the field experiment, seedling emergence was tested as a function of seed hydration treatment (as a factor) and included continuous microsite variables (vegetation cover, leaf litter, PAR, soil moisture, available water capacity, field capacity, permanent wilting percentage and bulk density). In the laboratory experiment, seed germination and seedling emergence were tested as a function

of the experimental factors (seed hydration treatment and soil moisture content) and their interaction. Statistical analyses were performed using the program R (v. 3.0.1. 2013). Hereafter, data are presented as mean values ±1 standard error (SE).

Results

Seedling emergence under field conditions

From the 1200 seeds sown, a total of 37 % emerged as seedlings and 19 % still survived at 11 months after sowing (February 2014–January 2015). The first seedling emergence was observed at 49–50 days after sowing. Contrary to expectation, the seedling emergence rate was higher in non-hydrated than in hydrated seeds (39 ± 0.25 vs. 34 ± 0.23 %; Log-rank = 36; *d.f.* = 1; $P < 0.001$; Fig. 1). The main causes of seedling mortality were desiccation (45 ± 0.30 %), physical damage by falling branches and leaf litter (40 ± 0.29 %) and undetermined (15 ± 0.16 %). Seedlings from hydrated and non-hydrated seeds presented similar mortality (49 ± 0.18 vs. 51 ± 0.19 %, respectively).

Initial microenvironmental and soil conditions were measured in each microsite (Table 1). To avoid correlation between microenvironmental and soil variables; we selected vegetation cover (%), leaf litter (%), PAR (%), soil moisture content (%), available water capacity (%) and permanent wilting percentage (%) for inclusion in a logistic regression model.

Seedling emergence was significantly affected by soil moisture and overhead vegetation cover (Table 2). We recorded more emerged seedlings in microsites with higher soil moisture but emergence decreased as vegetation cover increased (Fig. 2). After 11 months, seedlings reached 6 ± 0.37 cm in height and 1 ± 0.06 mm in basal stem diameter, with a total of 4 ± 0.26 leaves per seedling.

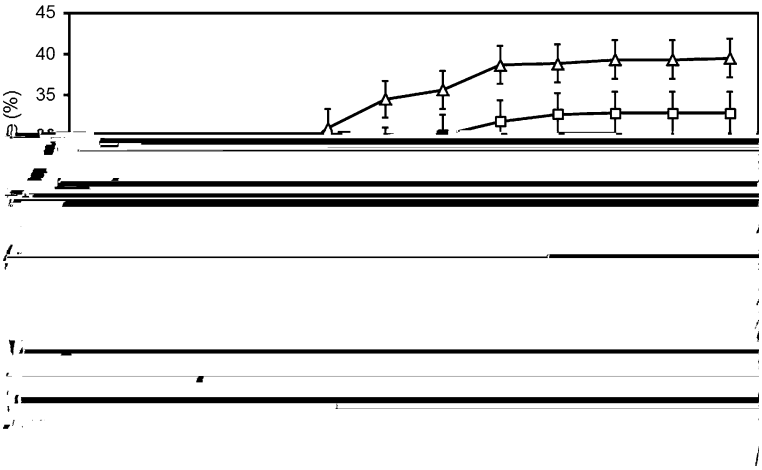

Fig. 1 Accumulated seedling emergence of *Oreomunnea mexicana* under field conditions during 2014 and early 2015. *Triangle symbol* represents seedlings from non-hydrated seeds; *square symbol* represents seedlings from hydrated seeds

Table 2 Results of logistic regression analysis of A) *Oreomunnea mexicana* seedling emergence under field conditions, affected by treatment (hydrated vs. non-hydrated seeds), vegetation cover at ground level (%) and soil moisture content in each microsite (%). B) Seed germination and C) seedling emergence under laboratory conditions are presented as a function of treatment (hydrated vs. non-hydrated seeds), soil moisture content (20–30 vs. 50–60 %), and their interaction, in seed germination and seedling emergence under laboratory conditions

Effect	B	±1 SE	Z value	Wald (χ^2)	*P*	Exp (B)
A. Seedling emergence under field conditions						
Treatment	−0.247	0.121	−2.049	4.198	0.040	0.780
Vegetation cover	−0.018	0.008	−2.265	5.130	0.031	0.981
Soil moisture	0.011	0.004	2.226	4.955	0.025	1.011
B. Seed germination under laboratory conditions						
Soil moisture	−1.777	0.204	−8.71	75.864	0.000	0.169
Treatment	−0.349	0.225	−1.554	2.415	0.1201	0.705
Soil moisture * treatment	−0.471	0.281	−1.674	2.802	0.0941	0.624
C. Seedling emergence under laboratory conditions						
Soil moisture	−4.650	0.330	−14.1	198.81	0.000	0.01
Treatment	−0.226	0.203	−1.111	1.234	0.266	0.798
Soil moisture * treatment	−0.194	0.506	−0.383	0.147	0.702	0.824

B = slope of logistic regression; SE = standard error; Wald (χ^2) = measure of significance of B; Sig. = significant *P* values (< 0.05); Exp (B) = odds ratio

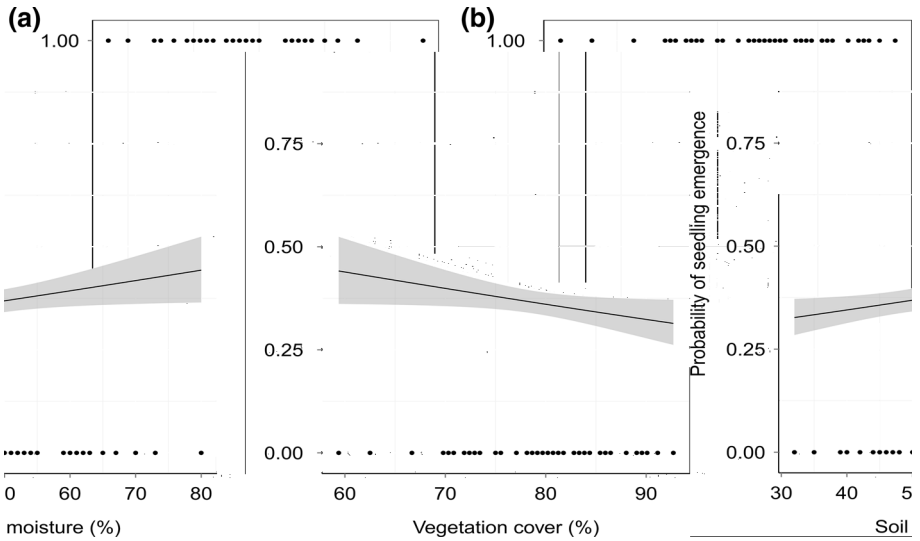

Fig. 2 Probability of seedling emergence as estimated by the GLM of *Oreomunnea mexicana* in field conditions per **a** soil moisture content (%) and **b** vegetation cover (%). *Gray area* represents the 95 % confidence interval

Table 3 Seed germination, seedling emergence and survival (% ±1 SE) of *Oreomunnea mexicana* as a function of treatment (hydrated vs. non-hydrated seeds) and soil moisture content (%) after 10 months under laboratory conditions

Treatment	Soil moisture	Germination	Emergence	Survival	Germinated seeds	Emerged seedlings	Seedling survival
Hydrated seeds	20–30	32 ± 0.41	2.67 ± 0.10	100 ± 0.00	96	8	8
	50–60	82 ± 0.27	78 ± 0.27	82 ± 0.34	246	234	191
Total hydrated		57 ± 0.05	40 ± 0.07	33 ± 0.06	342	242	199
Non-hydrated seeds	20–30	51 ± 0.49	4.33 ± 0.13	100 ± 0.00	152	13	13
	50–60	86 ± 0.25	82 ± 0.02	70 ± 0.32	259	246	171
Total non-hydrated		69 ± 0.05	43 ± 0.07	31 ± 0.05	411	259	184
Total	20–30	41 ± 0.34	3.5 ± 0.09	3.5 ± 0.09	248	21	21
Total	50–60	84 ± 0.19	80 ± 0.18	60 ± 0.24	505	480	362
Total		63 ± 0.27	42 ± 0.36	32 ± 0.29	753	501	383

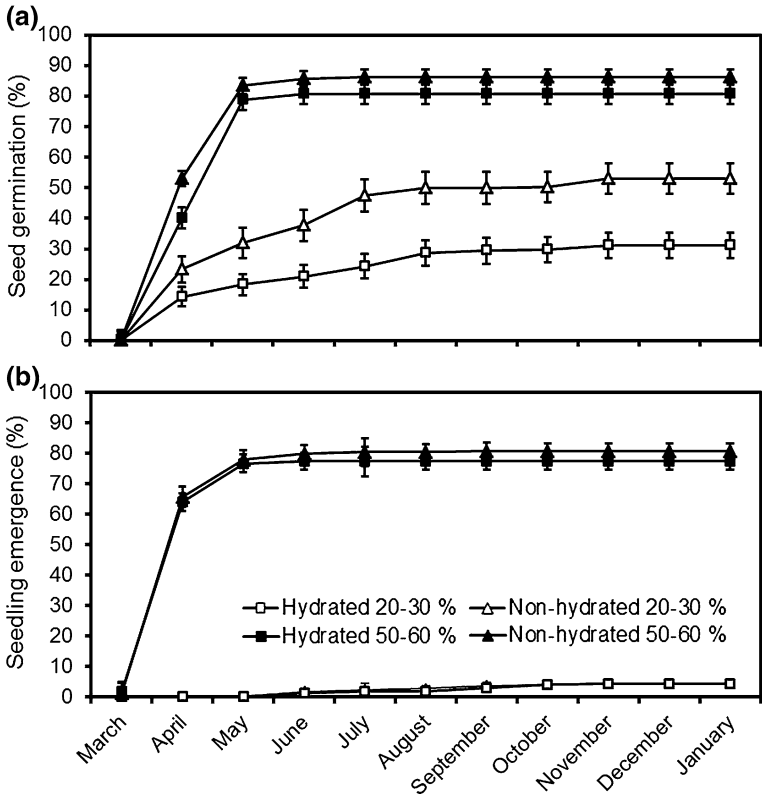

Fig. 3 **a** Seed germination and **b** cumulative seedling emergence (± 1 SE) of *Oreomunnea mexicana* under laboratory conditions as a function of treatment (hydrated vs. non-hydrated seeds) and soil moisture content (20–30 vs. 50–60 %) over a period of 10 months. Treatments with soil moisture content of 20–30 % overlap in seedling emergence

however, according to our results, seed hydration for 72 h had a negative effect on seed germination in *O. mexicana*. For *Magnolia dealbata*, the most successful pre-germination treatment was seed hydration for 24 h at room temperature, compared to 48 and 72 h with different temperature treatments (Corral-Aguirre and Sánchez-Velásquez 2006). A negative effect of soaking has also been observed in other tree species such as *Cordia megalantha*, which germinates rapidly but loses viability very quickly and is therefore not favored by a 24 h soaking period (Alvarado-López et al. 2014). While the duration of seed soaking was not specifically addressed in this study, it is a factor that could have played an important role in the results observed, as has been shown in other studies (Venudevan and Srimathi 2013). Another pre-germination treatment that should be explored is seed burial in wet soil, even for short periods, as this seems to promote tree seed hydration and protein mobilization (Benítez-Rodríguez et al. 2014).

Soil moisture and microsite variables effects on seedling emergence and survival

Our results suggest that soil moisture content is an important factor for germination, seedling emergence and survival. Germination was higher in seeds sown in soil with higher

moisture content (50–60 %) under controlled conditions, and in microsites with higher soil moisture under field conditions. An evaluation conducted in the field site found that, over one year, average soil surface moisture content ranges from 44 to 45 % and no great annual difference exists in these values (1.4–1.7 % of variation). In fact, during the studied period (2014), there was no evidence of soil water deficiency (Arreola-Flores 2016). Forest fragments with these slight variations in surface soil moisture conditions may provide suitable microsites for *O. mexicana*; however, this condition may also present high annual variation as a result of climate change.

The particular importance of soil moisture for tree seedling emergence of TMCF species has been reported for other tree species such as *Carpinus tropicalis*, *Liquidambar styraciflua* (Pedraza-Pérez and Williams-Linera 2005) and *Fagus grandifolia* (Álvarez-Aquino and Williams-Linera 2002). Ensuring adequate and constant moisture in the substrate can increase seed germination and emergence in these species. Vegetation cover was also an important factor dictating *O. mexicana* seedling emergence, which seems to be favored by a vegetation cover of 60–70 % at ground level. Avendaño-Yáñez et al. (2014) reported that *O. mexicana* presented higher survival under the vegetation cover of pioneer species compared to that found in open sites, while diameter and height growth was unaffected. This higher survival has also been observed under pine plantations compared to open sites although, in this case, seedling growth is lower than in the open sites (Avendaño-Yáñez et al.ompared 2016). This suggests that establishment could be favored by partially shaded microsites where there are few moisture and lower competition with the herbaceous str toring of the establishment and growth of this species is

Early seedling establishment is one of the most critica (Fenner and Thompson 2013). In this study, we did not find evidence of the presence of fungal infection or of seedling predation; however, these factors have been reported as an important cause of mortality in TMCF (in Veracruz) for *Carpinus tropicalis* and *L. styraciflua* (Pedraza-Pérez and Williams-Linera 2005).

Oreomunnea mexicana is considered to be at great risk because of potential climate change to drier conditions, while few populations remain in central Veracruz where its distribution range has been largely converted to human land uses (González-Espinosa et al.ompared 20 forms monospecific stands (Gonzá ability to establish vi where photosynthesis

Management implications

Compared to other restoration strategies, direct seeding implies lower economic costs, an opportunity to cover large areas (Tunjai and Elliott 2012) and the development of well-structured root systems once seedlings are established (Douglas et al. 2007). In particular, it has been documented that *O. mexicana* forms arbuscular mycorrhizal associations (Corrales et al. 2016a, b) that can enhance soil nutrient uptake. There is therefore a need to test the potential advantage of introducing inoculated seeds into degraded or secondary tropical forests soils. Our study shows that introducing *O. mexicana* through direct seeding is feasible since the seeds did not suffer any predation and the subsequent emergence of seedlings under field conditions reached 87 % of that recorded under optimal (laboratory) conditions. In fact, overall seedling survival of the species in this study was 46 %, which is similar to the values (40–62 %) previously recorded by Avendaño-Yáñez et al. (2014; over 24 months of monitoring) for 20-month-old *O. mexicana* seedlings planted under the canopy of pioneer species in Veracruz, Mexico.

This is the first study to suggest practices for the successful introduction of *O. mexicana* and acceleration of the recovery process of the secondary TMCF of the region. *Oreomunnea mexicana* has potential for introduction in secondary forests through direct seeding, but the highest probability of successful establishment would be achieved if the seeds are sown in sites with soil moisture above 50 % and an overhead vegetation cover of between 60–70 %. However, further research is required to determine if these particular microhabitat conditions are also optimal for seedling survival and sapling growth. Given the threatened status of the species; it is important to conduct studies that describe the frequency, synchrony and quantity of its fructification and to explore the genetic variability of the regional populations. Genetic and seasonal variability in seed quality should be considered in future studies, considering that the seeds utilized in the present study were only collected from two individuals. It is also necessary to consider that the maternal origin of the seeds could have a direct effect on their quality and therefore on germination and emergence. Other restoration techniques, such as seedling transplantation from natural seedling banks and introduction of nursery-propagated seedlings, could be explored and compared. Finally, there is a need for continued long-term monitoring of seedling survival and growth in order to evaluate the costs and benefits, both economic and ecological, of implementation of this and other methods and to assess the early establishment of this species and thus contribute to the knowledge regarding its natural regeneration and conservation.

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