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ORIGINAL PAPER

Effect of hydropriming and acclimation treatments on *Quercus rugosa* acorns and seedlings

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Abstract To facilitate restoration of the lava field forests surrounding Mexico City, we developed methods to improve the germination and field seedling performance of *Quercus rugosa* using hydropriming (regulated hydration of seeds in water), and we used special watering regimes to improve seedling acclimation. The size, dry mass, fresh mass and water content of seeds were measured, and curves were generated to evaluate acorn hydration and dehydration. The effects of stratification (5°C), heat shock (50°C) and scarification on germination were tested. All treated seeds and controls were germinated in control chambers at 21°C. One hydropriming cycle (PC) consisted of two hydration days followed by two dehydration days; treatments of 1, 2 and 3 PCs were tested. Seedlings from 1PC to 2PC were acclimated in a shade house under high and low watering regimes (400 and 200 mL week^{-1}, respectively). In the shade house and field, the effects of hydropriming and watering treatments were evaluated by measuring length, basal diameter, crown cover, number of leaves and branches and leaf area of seedlings. Dry and fresh mass were used to calculate acorn water content. Dehydration and hydration curves displayed hysteresis. Acorns exhibited physiological dormancy, which could be overcome by stratification or by 1 month of storage. 1PC led to increased germination rates and final germination. In both the shade house and field, 1PC showed a positive effect on all seedling growth parameters except branch number. Field survival was not affected. Generally, 1PC favoured efficient seed germination, seedling vigour and homogenous plant production.

Keywords Acorn germination · Netleaf oak · Priming cycles · Recalcitrant acorns · Seedling growth · Watering treatments

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Introduction

Global deforestation (13 million ha year^{-1}; FAO 2006) is the primary threat to this planet's ecosystems and species. There are two main methods for combating the detrimental effects of deforestation: preserving wild habitats through conservation and restoration, and preserving diversity by storing germplasms in seed banks. The latter is impracticable for species with recalcitrant seeds that quickly lose their viability. They cannot tolerate dryness and low temperatures, and consequently, they are not amenable seed bank storage (Hong and Ellis 1996; Finch-Savage and Farrant 1997; Daws et al. 2006). More information about the early life stages of recalcitrant species is needed as this will aid reforestation and restoration projects by enabling the development of strategies for successful germination, seedling growth and establishment.

Quercus is a widely distributed genus (300–531 species in the world) with two main diversity centres: Asia and Mexico. This genus comprises three groups: *Quercus*

(white oaks), *Lobatae* (red oaks) and *Protobalanus* (intermediate oaks). *Quercus* and *Lobatae* contain the majority of the species (Valencia 2004). Under optimal conditions, red oak seeds can be stored for at least 3–5 years, even at below-freezing temperatures (−2°C, Bonner and Vozzo 1987; Connor 2004). In contrast, white oak seeds can be stored for only 6–12 months, such that propagation must normally be performed immediately after collection (Bonner 1973, 2003). However, at low temperatures (1.6°C) and in storage conditions that avoid dehydration but favour aeration, acorns of the white oak *Q. garryana* have been shown to remain viable for 2 years (Devine et al. 2010).

Despite Mexico is considered the major centre of *Quercus* species diversity (Nixon 1998), Mexican oak forests have been allowed to become severely reduced. For this study, we selected the white oak *Quercus rugosa*, which grows in the forests surrounding Mexico City. Mexico City is one of the most populous cities in the world, and restoration has become an important goal for the region (Ingram 2008). The transition between seed to seedling life stages is very difficult for white oaks in disturbed environments. They exhibit high mortality due to seeds and seedlings dehydration, which occurs easily in some of the harsh environments where *Q. rugosa* occurs, such as lava fields (Bonfil and Soberón 1999). Thus, we focus on pre-germination treatments as a way to improve the germination, establishment and growth of *Q. rugosa* seedlings.

Our goal is to enable restoration efforts for *Q. rugosa* in Mexico and elsewhere in its distribution (from the southern United States to Guatemala and Honduras) and to contribute knowledge about the germination processes of white oak species in general. To that end, we tested a priming technique as a way to improve seed germination and seedling performance in disturbed areas. Priming treatments consist of seed hydration followed by dehydration, which enhances uniform germination, promotes early plant growth and survival, invigorates old seeds and increases biomass production under both optimal and limiting field conditions (Bray 1995; Sánchez et al. 2001, 2003). This germination pretreatment is usually applied in agronomic species by regulating hydration with osmotic solutions (osmopriming) or by regulating imbibition in water (hydropriming) or in a solid matrix (matripriming). Hydration by soaking is commonly used by scientists and nurserymen to enhance acorn germination (Bonner and Vozzo 1987; Bonner 2003). Soaking acorns in GA_3 and KNO_3 solutions, followed by immediate acorn sowing, produces a marginal enhancement of germination compared to other methods, such as acorn scarification (Struve 1998; Purohit et al. 2009). Prolonged soaking in PEG 6000 (polyethylene glycol) and water followed by immediate sowing (without dehydration) was shown to have a positive effect on acorn germination in *Q. rubra* (Struve 1998). We believe hydropriming (seed hydration in water followed by dehydration) and drought acclimation can improve seed germination and seedling growth and survival for *Quercus rugosa* and can be implemented as a forest restoration technique with ease and low cost.

The species

Quercus rugosa Née is a perennial white oak. In some years, a fraction of the acorns produced are viviparous (the radicle protrudes when acorns are still on the tree). This occurred during the year of this study, but in others years, the acorns remain in different levels of dormancy (from superficial, where the seeds loose dormancy during storage, to relatively deep, where acorns require pregerminative treatments to germinate). Even in years marked by dormancy, there are some seeds that can germinate during storage. Non-viviparous seeds can be stored for 3 months while maintaining their viability (Vázquez-Yanes et al. 1999). Flowering occurs from March to June, and seed dispersal occurs from October to February (Vázquez-Yanes et al. 1999). *Quercus rugosa* is considered a useful species for restoration ecology programs because it can improve ecosystem productivity; it incorporates nutrients into the soil through litter decomposition, preventing soil erosion and helping to preserve and recharge the city aquifers (Vázquez-Yanes et al. 1999; Soberón et al. 1991).

Study area

The study area is located on the Ajusco mountain to the south of Mexico City in the Parque Ecológico de la Ciudad de México (PECM; 19°10′N and 99°13′W), which was designated as a protected area in 1989 (Soberón et al. 1991). It covers 727 ha and is situated at 2,360–2,860 m above sea level (asl), which provides a moist and temperate climate (González-Hidalgo et al. 2001). The mean annual precipitation is 803 mm, and the mean annual temperature is 15.5°C (Calderón and Rzedowski 2001). In the Ajusco, *Q. rugosa* grows from 2,500 to 3,150 m asl on lava fields that formed 1,670–2,000 years ago after the eruption of the volcano Xitle (Siebe 2009). Primary succession on the lava fields has been severely disrupted in a 200 ha area by human settlement, which mainly occurred between 1982 and 1990 (Soberón et al. 1991). Lava substrate and soil have been removed in some zones, and we used one such area to transplant seedlings of *Q. rugosa*. This area is a secondary open xerophilous shrubland with some small, isolated *Q. rugosa* trees. The volcanic rock is visible at the surface with only scant soil accumulation on top. This disturbed area is surrounded by pure and mixed stands of

Q. rugosa, but there is very little recruitment of seedlings to the disturbed area from the surrounding stands.

Materials and methods

Size, dry and fresh mass and acorn water content

In November 2004, at the beginning of the dry season, acorns of *Quercus rugosa* were directly collected from more than 20 trees growing in the PECM. After collection, the acorns were exposed to petroleum ether (J. T. Baker, México) vapour for 30 min to eliminate insects and larvae. This chemical does not have adverse effects on the acorns.

After field collection, the acorns were placed in paper bags and transported to the laboratory. Seed characteristics were evaluated using 30 acorns. Size was quantified by measuring length and width. The fresh mass was determined on the collection day with a balance from OHAUS (Brainweigh B 300D, USA, ±0.007/0.001 g). The dry mass was obtained after acorns were cut into two parts and dried for 7 days at 70°C in an oven (Riossa, México). The acorn water content (WC) was calculated as a percentage of the dry mass using Eq. 1:

$$\mathrm{WC_{db}}(\%) = [(\text{fresh weight} - \text{dry weight}) \div \text{dry weight}] \times 100 \quad (1)$$

and as a percentage of the fresh mass using Eq. 2:

$$\mathrm{WC_{wb}}(\%) = [(\text{fresh weight} - \text{dry weight}) \div \text{fresh weight}] \times 100. \quad (2)$$

To obtain a useful parameter to infer acorn water content, we related the dry mass, fresh mass and $\mathrm{WC_{db}}$ by regression analysis.

Hydration and dehydration rates

Acorn dehydration rates were estimated immediately after collection with 4 different humidity and temperature treatments: (1) 5°C on a dry surface, (2) 21°C on a dry surface, (3) 21°C on a wet agar surface (1% in water) and (4) 21°C following a 7-day hydration period. For each treatment, 30 acorns were used. Temperatures of 5°C and 21°C simulated the mean minimum and maximum temperatures that occur during acorn germination periods at the end of the rainy season and beginning of the dry season (autumn and early winter, respectively; Fig. 1).

For hydration rate estimation, 30 acorns were weighed and then placed in water to cover 75% of each acorn, with the micropylar region kept exposed to air. Every 4 h during the first 12 h, each acorn was taken out of the water, blotted with a paper towel and weighed. Subsequently, the acorns were weighed every 12 or 24 h up to a maximum of 643 h (26 days), depending on the imbibition rate. Afterwards, the acorns were dried in an oven (as described above) to obtain the dry weight. The $\mathrm{WC_{db}}$ percentages were arcsine transformed and fitted to the exponential function (Eq. 3):

$$y = a + b^{(-x/c)}. \quad (3)$$

The hydration and dehydration rates were obtained from the first maximum and minimum derivatives of the curves, respectively. The effects of the treatments on the dehydration rates, final $\mathrm{WC_{db}}$ and final relative water content, which was calculated as (Eq. 4):

$$\text{Relative WC} = [(\text{initial WC}_{\mathrm{db}} \times 100) \div \text{final WC}_{\mathrm{db}}] \quad (4)$$

were analysed by a Kruskal–Wallis test and visually compared with box-and-whiskers plots.

Initial germination tests

Germination was tested after applying one of the following treatments: (1) scarification of acorns (two longitudinal cuts made in the acorns with a knife, (2) incubation of acorns at 50°C for 2 h, (3) stratification of non-imbibed acorns at 5°C for 7 days, (4) storage at 5°C for 26 days and (5) no treatment (control group). Seeds were germinated in boxes (23 × 17 × 4 cm) on an agar surface at 21°C. Cumulative germination was fitted to a sigmoid function (Eq. 5):

$$Y = a \div [1 + (bx^c)]. \quad (5)$$

The germination rate was calculated as the first maximal derivative of the curve. All germination treatments had three replicates with 30 acorns each. The final germination percentage, lag time (time to initiate germination) and germination rate were compared among treatments with the Kruskal–Wallis test.

Hydropriming treatments

Before hydropriming treatments, all acorns were individually weighed. During the hydropriming treatments, the acorns were weighed every 24 h to evaluate acorn water uptake. Dry mass was calculated from the regression equation relating the fresh mass and dry mass of the acorns used to estimate the acorn water content (see above). ANOVA tests were used to assess the effects of treatments on the final $\mathrm{WC_{db}}$ after log-transformation of this response variable to meet normality criteria.

For hydropriming treatments, we selected a mild dehydration because the seeds of *Q. rugosa* are recalcitrant. Scarified seeds were exposed to hydropriming treatments

consisting of hydration-dehydration cycles. One hydropriming cycle consisted of two hydration days in water followed by two dehydration days on a dry surface at 21°C. The treatment groups received one (1PC), two (2PC) or three hydropriming cycles (3PC). Control (C) acorns were scarified but not primed.

After treatment, the acorns were arranged in groups of thirty and sown inside closed-top plastic boxes (23 × 17 cm × 4 cm) with wet coarse sand as a substrate. Each treatment had three replicates. Boxes were placed in controlled environment chambers (model 844, Lab-line Instruments, Inc., Melrose Park, IL, USA) at a constant temperature of 21°C with a 12-h light photoperiod.

An acorn was considered to be germinated when the radicle protruded by 5 mm. Germination progress was recorded every 3 days. Germination percentages were arcsine transformed and fitted to sigmoid curves. The germination capacity, germination rate and lag time were compared with a Kruskal–Wallis or an ANOVA test, depending on the homogeneity of variance and data normality (Zar 1974).

Seedling acclimation treatments

Seedlings that were ~65 days old and that emerged from the 1PC, 2PC (both at 21°C) and control treatments were individually transplanted to black plastic bags (23 × 50 cm) with a soil:sand substrate (1:1 v/v). In a shade house, half of the seedlings from each treatment were exposed to a high watering treatment (HW, 400 mL week^{-1}), and the other half were exposed to a low watering treatment (LW, 200 mL week^{-1}), resulting in six combinations of hydropriming and watering treatment conditions. Watering treatments were determined previously in three seedlings that were 50 days old, which were sown individually in a pot and watered at field capacity. These pots were weighed at the beginning and after 15 days, when seedlings reached the permanent wilting point. At this time, seedlings were ~105 days old. At the beginning of the wet season (July 2006), 13 seedlings from each of the six acclimation treatments were measured to obtain total length, basal stem perimeter, crown diameter, number of leaves and branches and leaf area. Then 21 seedlings per treatment were transplanted to the disturbed site of the PECM described above. Fifteen days after transplanting, the growth variables were measured again, and this was repeated every 30 days until December 2006, when the seedlings were 375 days old. Survival was also recorded during each evaluation.

The basal stem diameter and the seedling cover were calculated from the stem perimeter and the diameter of the crown, respectively, by assuming a circular shape for both traits.

The leaf area of 15 leaves was measured with an area foliar meter (LI-COR, 3000 A, Lincoln, NE, USA). The individual leaf area of 30 leaves was related to leaf length by regression analysis, and the resulting model is given by Eq. 6:

$$Y = a + bx^c. \quad (6)$$

Equation 6 was used to calculate individual leaf area from leaf length, and the individual leaf areas were used to calculate total foliar area. Analysis of the effect of acclimation treatments on seedling growth and survival achieved up to the last census date were conducted with ANOVA and logistic regression analysis, respectively (JMP ver. 8 Software SAS Institute Inc., Cary, NC, USA).

Results

Size, dry and fresh mass and acorn water content

The fresh and dry masses of acorns were tightly linearly related ($r^2 = 0.90$, $F_{29} = 257.1$, $P = 0.00001$; $y = 0.16 + 0.508x$). Neither fresh nor dry mass had a strong relationship with the acorn WC_{db}. The mean fresh mass was 4.29 ± 0.24 g, and the mean dry mass was 2.34 ± 0.13 g. The mean WC_{db} was 83.35 ± 3.45%, and the mean WC_{wb} was 45.32 ± 1%.

Acorn hydration and dehydration rates

The maximal instantaneous hydration rate was found in acorns previously placed in contact with water for 7 days (524.91 ± 39.82 g h^{-1}), and the final WC_{db} in these seeds was 90.82 ± 1.19% (Fig. 2a). The final WC_{db} and the dehydration rate of acorns exposed to the dehydration

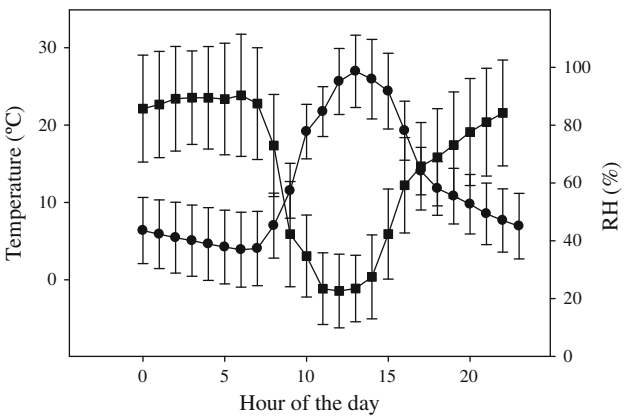

Fig. 1 Daily temperature (*filled circle*) and relative humidity (*filled square*) during November, December and January of 2005–2006, in a forest edge of the Parque Ecológico de la Ciudad de México. Mean values ± SD are shown ($n = 100$)

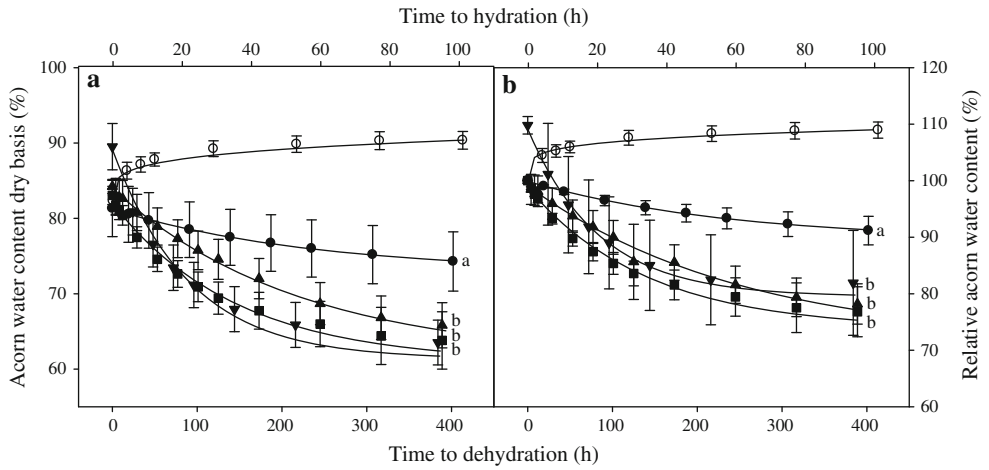

Fig. 2 Time course of: **a** relative water content and **b** absolute water content (dry mass basis), during hydration (*open circle*) and dehydration of acorns of *Q. rugosa* carried out in four conditions: (*filled circle*) dehydration at 5°C on a dry surface, (*filled square*) dehydration at 21°C on a dry surface, (*filled triangle*) dehydration at 21°C on a wet agar surface, (*filled inverted triangle*) dehydration at 21°C on a dry surface after acorns were immersed in water for 7 days. Mean values ± 2 EE are shown ($n = 3$). *Letters* indicate statistical differences

treatments differed significantly among them ($H = 37.92$, $P = 0.0001$ and $H = 88.48$, $P = 0.0001$, respectively). The dehydration rate of acorns stored at 5°C was the slowest, and their final WC_{db} was the highest among the treatments (Fig. 2). The highest dehydration rate occurred in seeds previously hydrated for 7 days and dehydrated at 21°C (Fig. 2). These seeds also had the lowest WC_{db}, but the difference was not significant when compared to the other treatments. The relative WC_{db} reached by acorns in these treatments is shown in Fig. 2b.

Initial germination tests

The final germination percentage and the lag time were significantly different between the germination treatments ($H = 9.84$, $P = 0.04$ and $H = 11.25$, $P = 0.02$, respectively). However, there were no differences in germination rate ($H = 8.96$; $P = 0.06$). Acorns exposed to 5°C for 1 week exhibited the maximum germination percentage. Germination began earlier in the scarified seeds and in seeds previously heated at 50°C (2 h). Control seeds and seeds that were pretreated at 5°C for 1 month had the longest germination lag times (10–14 days, Fig. 3). Scarified acorns showed the highest germination variability.

Hydropriming treatments

The hydration course (Fig. 2) determined the hydration and dehydration lengths during the hydropriming treatments. After 2 days, the hydration curve was asymptotic, and the dehydration of acorns previously hydrated for 7 days had a

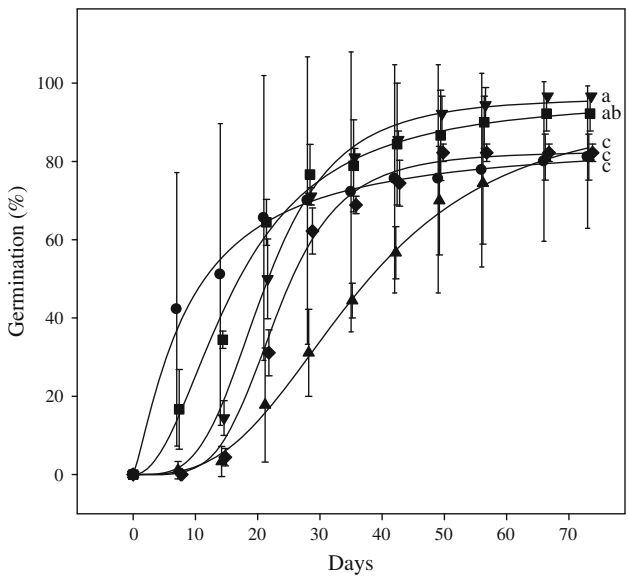

Fig. 3 Time course of cumulative germination percentage after acorns were exposed to: (*filled circle*) mechanical scarification, (*filled square*) 50°C for 2 h, (*filled inverted triangle*) 5°C for 1 week, (*filled rhombus*) 5°C for 1 month, (*filled triangle*) control. Mean values ± 2 EE are shown ($n = 3$). *Letters* indicate statistical differences

WC_{db} 3% lower than the initial W_{db}. However, the hydration course during each hydropriming cycle showed that acorns reached and maintained a higher WC_{db} after each subsequent hydropriming cycle (Fig. 4). The results showed that after each PC treatment, the acorn WC_{db} did not return to the WC_{db} measured before applying the PC treatment. 2PC and 3PC had a similar effect, such that the higher WC values achieved after the hydration period

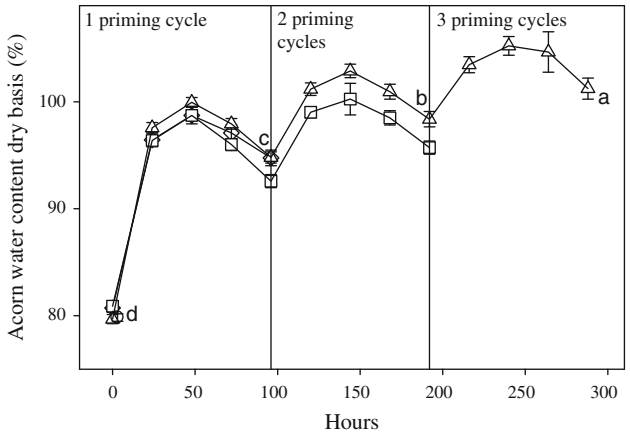

Fig. 4 Temporal course of water content (dry basis) of acorns subjected to different hydration-dehydration cycles: (*open circle*) control, (*open inverted triangle*) 1 cycle, (*open square*) 2 cycles, (*open triangle*) 3 cycles. Each hydropriming cycle consisted of two hydration days followed by two dehydration days. The net increment in WC_{db} after each dehydration and dehydration period is shown. Mean values ± 2 EE are shown ($n = 3$). *Letters* indicate statistical differences

for each PC could be described by the function $Y = 80.79 + 1.03(x^{0.52})$, whereas the increase in retained water at the end of each PC (after dehydration) could be described by the function $Y = (81.07 + 21.82x^{0.5})/(1 + bx^{0.5})$.

Hydropriming treatments had significant effects on the final germination percentage ($F_{(3,11)} = 5.95$; $P = 0.002$), germination rate ($F_{(3,11)} = 6.33$; $P = 0.016$) and lag time ($H = 0.8.55$; $P = 0.035$). 1PC had the greatest germination success after 6 days (96.66 ± 6.67%); 2PC, 3PC and the control group required 9–18 days (Fig. 5). The control group and 3PC showed similar germination percentages, which were significantly lower than that of 1PC.

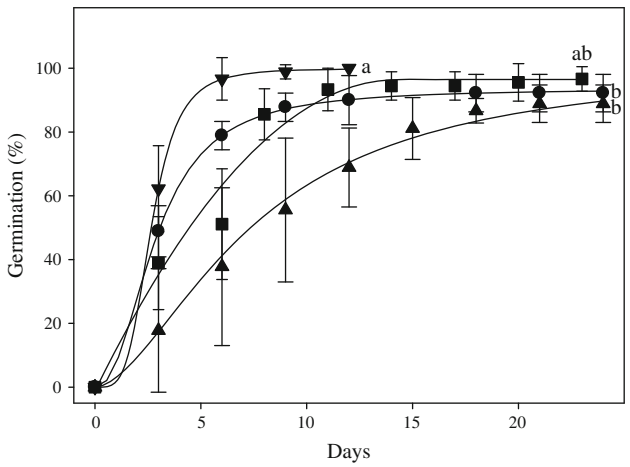

Fig. 5 Time course of cumulative germination percentage at 21°C, after which acorns were exposed to the following treatments: (*filled circle*) control, (*filled inverted triangle*) 1 hydropriming cycle, (*filled square*) 2 hydropriming cycles, (*filled triangle*) 3 hydropriming cycles. Mean values ± 2 EE are shown ($n = 3$). *Letters* indicate statistical differences

Seedlings acclimation treatments

At the end of the rainy season (December 2005), seedling height was found to vary significantly among different acclimation treatments ($F_{(5,75)} = 12.1$, $P < 0.00001$; Fig. 6a), leaf area ($F_{(5,75)} = 9.09$, $P < 0.00001$; Fig. 6b), as did basal stem diameter ($F_{(5.75)} = 3.19$, $P = 0.011$; Table 1) and seedling cover ($F_{(5.75)} = 5.92$, $P < 0.00001$; Table 1). Seedlings receiving HW treatments had the highest growth values. The control group with LW treatment had the lowest values for all growth variables. The seedlings coming from

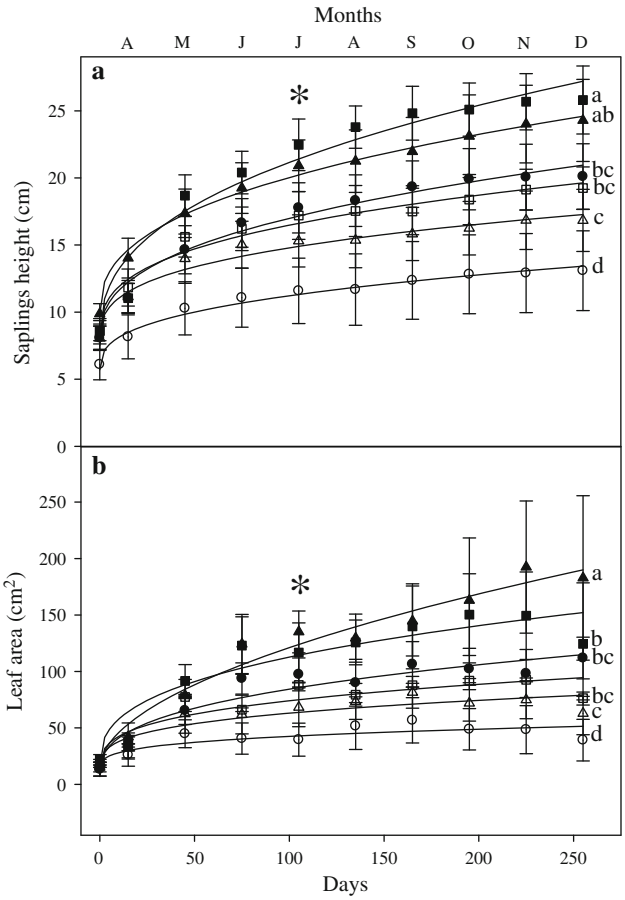

Fig. 6 Time course of growth in the field, expressed as: **a** height and **b** leaf area of seedlings from acorns with hydropriming treatments: (*filled circle*, *open circle*) control; (*filled square*, *open square*) 1 priming cycle; (*filled triangle*, *open triangle*) 2 priming cycles. Each hydropriming cycle consisted of two hydration and two dehydration days. Previous to transplanting seedlings to the field, seedlings were exposed to different watering treatments in a shade house: high watering (*black symbols*) and low watering (*white symbols*). Mean values ± 2 EE are shown ($n = 10$). *Capital letters* indicate the months in which growth was measured, *the time when the plants were transplanted to the field, and *lowercase letters* indicate statistical differences

Table 1 Mean final growth values (±2 EE, $n = 20$) of seedlings transplanted to the Parque Ecológico de la Ciudad de México

Treatment	Basal diameter (cm)	Seedlings cover (cm^2)
Control LW	0.361 ± 0.114 b	47.93 ± 20.53 b
Control HW	0.489 ± 0.088 a	130.24 ± 33.98 a
1PC LW	0.511 ± 0.067 a	146.84 ± 44.37 a
1PC HW	0.568 ± 0.08 a	137.013 ± 38.06 a
2PC LW	0.459 ± 0.06 a	100.98 ± 20.24 a
2PC HW	0.557 ± 0.09 a	166.12 ± 55.81 a

The number that precedes PC indicates the number of the consecutive PCs applied to the acorns. Letters indicate significant differences between treatments

LW = watering with 200 mL $week^{-1}$, HW = watering with 400 mL $week^{-1}$, PC = priming treatment consisting of two acorn hydration days followed by two dehydration days

the primed acorns tended to have the highest values in both watering treatments. The 1PC-HW treatment produced the highest seedling growth in terms of height, and it was significantly different from the control treatments. The 2PC-HW treatment had the highest seedling leaf area. Seedlings in the control-LW treatment had significantly lower basal stem area and cover compared to all other treatments. After 195–365 days, survival did not differ significantly among treatments ($P > 0.05$); however, treatments with low watering tended to exhibit larger variation in survival among replicates than did treatments with high watering, and 1PC-HW showed the lowest variability. 2PC-HW also tended to yield the lowest survival rates (Fig. 7).

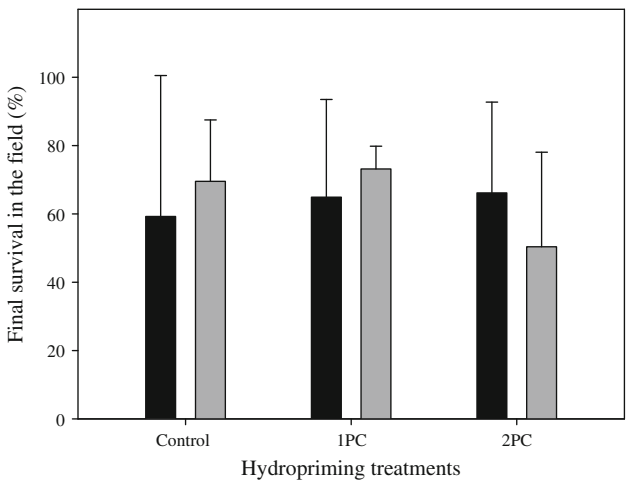

Fig. 7 Mean final survival of the saplings introduced to the field (±2 EE) pretreated with two watering levels. *Black bars* = watering with 200 mL $week^{-1}$, *grey bars* = watering with 400 mL $week^{-1}$, PC = priming treatment consisting of two acorn hydration days followed by two dehydration days. The number that precedes PC indicates the number of the consecutive PCs applied to the acorns

Discussion

Seed water content and germination

At the time of dispersal, *Quercus rugosa* acorns had $WC_{db} = 83.35\%$ (45.6% of WC_{wb}), which is similar to other *Quercus* species (Bonner 2003; Xia et al. 2010). After full hydration, acorns only increased their WC_{db} by 8.96% ($WC_{wb} = 8.22\%$). The low water uptake during imbibition is related to the recalcitrant nature of acorns. These seeds can germinate during storage (Bonner 1973; Devine et al. 2010; Purohit et al. 2009; Pritchard et al. 2004); however, an additional water uptake during soaking treatments enhances acorn germination (Bonner 2003). *Q. rugosa* acorns germinated during storage, and viviparous seedlings were observed growing on the trees. It has been reported that vivipary in *Q. virginiana* is associated with rain occurring during seed development (Bonner and Vozzo 1987), but the study year was not particularly rainy or warm.

The water content after 26 dehydration days was also very high, indicating high water retention in white oak acorns due to the abundance of highly hygroscopic starch in the cotyledons (Bonner and Vozzo 1987; Bonner 2003) and due to the waxy and coriaceous pericarp surrounding the seed (Bonner 1968). It has been reported that the acorns of white oaks can be viable in different proportions when the water content is between 30 and 50% (Schopmeyer 1974). After one month, acorns of *Quercus robur* (white oak) maintained only 60% of viability after adjusting the WC from 46 to 43% (Özbingöl and O'Reilly 2005). In this study, all dehydration treatments resulted in $WC_{db} \geq 60.5\%$ ($WC_{wb} \geq 37.7\%$). The dehydration rate was slow at 5°C, suggesting a slow dehydration inside the forest at 2,800–2,900 m asl, where temperatures are low during seed dispersal in late autumn and early winter (Fig. 1) (González-Hidalgo et al. 2001). Seeds dehydrated at 5°C on an agar surface for 1 month maintained 73. 4% of WC_{db} ($WC_{wb} = 43.3\%$), and 96% of them germinated at 21°C. Thus, inside the forest, acorn viability might be maintained for longer periods than have been reported for *Q. rugosa* (3 months according to Vázquez-Yanes et al. 1999) or were observed in this study (i.e. 6 months, A. Orozco-Segovia, personal observations). It has also been reported that at 5 and 7°C, 34% of *Q. rugosa* acorns can maintain viability for 11 months, but 44% germinate within that time (Zavala-Chávez 2004).

After one dehydration month, the acorns placed on the agar surface at 21°C reached 64% of WC_{db} ($WC_{wb} = 39\%$), similar to that of acorns incubated at the same temperature without contact with a wet surface. This result suggests that direct sowing is not an adequate restoration technique because day field temperatures in disturbed areas

and forest edges are higher than 21°C, even in the rainy season. It has also been reported that, in a shade house, *Q. rugosa* acorns receiving daily watering remain viable on the soil for only 35 days (Robledo 1997). Nevertheless, in disturbed sites, 46% of acorns germinate if protected from dehydration inside cages and covered by litter (Bonfil and Soberón 1999).

The change in the WC_{db} of the acorns exposed to dehydration or hydropriming treatments was calculated from the relationship between fresh and dry mass because fresh mass masked the acorn WC, as occurs in other species (Daws et al. 2004; Sánchez-Coronado et al. 2007). This relationship is useful for acorn management because WC can be estimated easily in a large acorn sample.

Hydration rates were highest within the first 2 days, but after that, water uptake was a slow process. Acorns of several species reach full imbibition after 2–5 days (Özbingöl and O'Reilly 2005; Purohit et al. 2009). The dehydration curves showed hysteresis (these curves had a rate-independent memory). That is, for acorns hydrated for 7 days (Fig. 2) and acorns hydrated for 2 days (Fig. 4), with subsequent two-day dehydration, the dehydration curves did not follow a course that was the inverse of the hydration curves. Consequently, the time needed for acorns to return to their initial water content was different in each case. Hysteresis has also been observed after one dehydration-hydration cycle in seeds of certain tropical tree species with high initial water content (Rodríguez et al. 2000). For *Q. rugosa*, such hysteresis was a disadvantage for the acorns under the 3PC treatment, which showed slow and asynchronous germination. This result suggests that germination had begun in some acorns; those embryos can become damaged when they are not able to continue the germination process (Bradford 1995). The increase in germination time in 3PC acorns may indicate that a period of time is required to repair the damage caused by dehydration (Vertucci and Farrant 1995; Boubriak et al. 1997). Treatments 2PC and 1PC improved germination, and we therefore recommend only the short hydration-dehydration periods provided by 1 and 2 PCs for *Q. rugosa* acorn management. Treatments reported previously contain the elements of priming applied in a different sequence. Dehydration alone, dehydration followed by soaking, and hydration followed by dehydration and cold storage all increase germination percentages and germination rates for *Q. robur* acorns (Özbingöl and O'Reilly 2005; Doody and O'Reilly 2008). These results and ours encourage further exploration of the effects of hydropriming treatments on acorn germination.

Twenty days after sowing, the germination percentages in the 3PC treatment group and the control group were similar. In contrast, acorns in the 1PC treatment reached 100% germination after only 6 days. On the forest floor, when rains occur daily (end of summer, beginning of autumn), mild acorn hydration and dehydration might concentrate germination within a short time period. As a consequence, seedlings might be well established before the beginning of the dry season, preventing acorn depredation by rodents and insect larvae (Xiao et al. 2009). However, during the dry season, even entire seedlings (5 months old) can be consumed by gophers when there is an overall shortage of food (personal observations).

Dormancy is uncommon among white oaks, but some species from the northern United States do exhibit dormancy (Bonner and Vozzo 1987). The long germination time (2 months, Fig. 3) exhibited by the control acorns of *Q. rugosa* suggests the presence of a non-deep physiological dormancy (sensu Baskin and Baskin 2004) (controls, Fig. 3). This non-deep physiological dormancy was broken in acorns exposed to stratification at 5°C for 1 week or given a shorter exposure (2 h) at 50°C. Much like the former case, a short period of acorn exposure to low temperatures may occur inside the forest or at the forest edge during dispersal, favouring germination. The latter case may be relevant to acorns exposed to high temperatures in unprotected sites in periods of peak sunshine. The exposure of acorns to sun for 6 h reduces germination in the white oak *Q. leucotrichophora* (Purohit et al. 2009). This might explain the infrequent establishment of *Q. rugosa* in open sites in the PECM. The favourable effect of high temperatures has been reported for red oaks (Abrams 2003; McPherson 1992), but not for white oaks. In the studied species, exposure to 50°C for 2 h can be useful to reduce the nursery care time required.

Scarified acorns exhibited the largest variation in germination (Fig. 3). This wide variation explains the lack of significant differences in the germination rate among treatments shown in Fig. 3. Making a cut in the chalazal region is the best way to increase germination for *Q. glauca* and *Q. leucotrichophora* (Purohit et al. 2009). In fact, after 2 weeks of storage (control, Fig. 5), scarification did not negatively affect the germination of *Q. rugosa*; instead, it encouraged germination during the soaking treatments.

Seedling acclimation

Seedling growth was reduced in acorns receiving low watering regardless of hydropriming treatment. The observed retardation of growth results from restriction of shoot elongation and foliar expansion due to partial water stress (Jones 1992). After transplantation to the field, there were no significant effects of the acclimation treatments on the growth parameters measured in this study, even during the months of the dry season (October, November and December). The seedlings exposed to LW did not show a better performance under transplanting stress and field

environment heterogeneity than did seedlings subjected to HW. The lack of observable differences between LW and HW seedlings may be due to the fact that all of these treatment groups were transplanted to the field in the rainy season, and field acclimatisation to endure dryness occurred for all the seedlings. An effect of acclimation on seedling growth has not been found in Mediterranean *Quercus* species, and this has been explained as the adaptation of these species to drought (Pausas et al. 2004). However, hydropriming treatments had a positive effect on all the growth parameters evaluated, with the exception of branch number. The 1PC priming type was the best pretreatment under either watering regime, and this effect was observed in both the shade house and in field conditions. Similar results have been found in other native species useful for restoration (Sánchez et al. 2001, 2003). During the study period, there were no significant differences in survival among treatments, which was very likely due to the small sampling size and the high environmental heterogeneity.

Conclusions

In conclusion, hydropriming (hydration-dehydration cycling) is a low-cost technique that is easy to apply and favours rapid, synchronous germination of *Q. rugosa* acorns. It improves plant growth in shade houses and in the field without affecting seedling survival. These favourable effects on germination and seedling growth have been previously reported for cultivated plants (Fujikura et al. 1993) and a handful of native tree species (Thanos and Skordilis 1987; Sánchez et al. 2003; among others). The adaptation of successful agricultural practices to restoration projects is an approach that merits exploration; many agricultural techniques might be quite useful in a restoration context.

To reduce *Q. rugosa* nursery care requirements, we recommend storing acorns at 5°C for 1 week before germination. Acorn dehydration can be controlled more easily at low temperatures, allowing acorn viability to be maintained for up to 1 month while avoiding germination during storage. This approach has also been demonstrated for other *Quercus* species (Bonner 2003). We recommend applying one cycle of priming treatment (two hydration days, followed by two dehydration days) as a restoration practice to shorten the time needed to complete germination. This treatment also improves seedling growth, which shortens the time required for plant production and outplanting to the field.

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