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RESEARCH PAPER

Effects of seed burial on germination, protein mobilisation and seedling survival in *Dodonaea viscosa*

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ABSTRACT

Ecological restoration of disturbed areas requires substantial knowledge of the germination of native plants and the creation of novel methods to increase seedling establishment in the field. We studied the effects of soil matrix priming on the germination of *Dodonaea viscosa* seeds, which exhibit physical dormancy. To this end, we buried both pre-scarified (in H_2SO_4, 3 min) and non-pre-scarified seeds in the Parque Ecológico de la Ciudad de México. After seeds were unearthed, they were post-scarified for 0, 2, 6 and 10 min and their germination percentages compared to the germination of a control batch of laboratory-stored seeds. For both control and unearthed seeds, the protein pattern was determined in the enriched storage protein fraction in SDS-PAGE gels stained with Coomassie blue. Percentage germination increased as the scarification time increased. Pre-scarification significantly increased percentage germination of post-scarified seeds in relation to the control and non-pre-scarified seeds. In seeds unearthed from the forest site, the buried pre-scarified seeds had relatively high percentage germination, even in the absence of post-scarification treatment. A 48-kDa protein was not found in unearthed, pre-scarified seeds nor in the control germinated seeds, indicating that mobilisation of this protein occurred during soil priming. Burying seeds for a short period, including the beginning of the rainy season, promoted natural priming, which increased protein mobilisation. Functionally, priming effects were reflected in high percentage seedling survival in both the shade house and the field. Seed burial also reduced the requirement for acidic post-scarification.

INTRODUCTION

Ecosystem restoration is urgently required to reverse the effects of high deforestation rates around the world, including the immediate consequences of soil erosion and flooding, even in urban areas (Bullock *et al.* 2011). These calamities have been associated with global climate change (FAO 2011). To restore deforested and depauperate ecosystems, it is necessary to create a species palette that includes native species, as well as pioneers and species from mature ecosystems (Mendoza-Hernandez *et al.* 2013a). Once the disturbance threshold has been identified (Briske *et al.* 2005), the next important step is to find the species that may have a facilitation role in the natural regeneration of vegetation. *Dodonaea viscosa* is an important facilitating species in lava fields that can help to accelerate recovery of the lava field shrublands (Mendoza-Hernandez *et al.* 2013a).

Substantial information concerning the germination of native species in tropical and subtropical areas is lacking; thus, it is necessary to generate such information (Meiners *et al.* 2002). Nevertheless, the main constraints to restoration are the fragility of the first two steps of plant life: germination and seedling establishment (Leck *et al.* 2008). A review of the agricultural tools used to improve seed germination percentages and, in turn, crop yield shows that priming treatments of regulated hydration followed by dehydration (to avoid radicle protrusion) are successful methods that enhance seed and seedling performance (Karseen *et al.* 1990; Halmer 2004). Seed priming is typically applied in one of three ways: (i) imbibition in an osmotic solution (osmopriming), (ii) seeds hydrated in water (hydropriming) or (iii) hydration in a solid matrix (*e.g.* vermiculite, in a process known as matrix priming). All of these pretreatments culminate in seed dehydration and storage. As a result of these pretreatments, germination is rapid and synchronous and seedling tolerance to stress is higher than in untreated seeds (Bray 1995; Sánchez *et al.* 2001). In seeds found within soil, priming treatments may also occur through variations in soil hydration. For example, the tropics may experience unpredictable winter rain pulses during the dry season, erratic rain preceding the rainy season, or encounter dry spells during the rainy season (Engelbrecht *et al.* 2006). Intermittent increases in soil moisture result in changes to seed hydration that do not necessarily conclude with radicle protrusion, but rather may allow the biochemical and functional processes that precede germination to occur (Bray 1995). These occurrences in the soil have been related to natural priming, and their functional results are similar to those reached through laboratory priming treatments (González-Zertuche *et al.* 2000, 2001; Gamboa-deBuen *et al.* 2006). One of the biochemical changes

that occur during priming is the mobilisation of seed storage proteins, mainly of globulins. These and other changes, such as synthesis of stress proteins, *e.g.* late embryogenesis abundant proteins (LEA; Bray 1995), represent early germination processes. In consequence, when seeds are rehydrated, germination occurs rapidly and synchronously and seedlings may be more tolerant to dry spells.

Application of priming treatments to seeds of native species may be the key to increasing seed germination and seedling recruitment for the restoration of native species in depauperate ecosystems. With this goal in mind, we tested the effects of natural priming on seeds of the pioneer tree *D. viscosa*. This species is distributed worldwide and is a common pioneer in disturbed areas of oak and pine forests (Mendoza-Hernández *et al.* 2013b). Hence, *D. viscosa* is one of the main species propagated in commercial shade houses for use in reforestation (Camacho *et al.* 1993). However, field survival is relatively high only in areas with deep soils (Camacho 2003), but in shallow or scant soils, such as in lava fields, survival is very poor or absent (Pedrero-López 2011). Additionally, in the nursery there is high seedling mortality caused by damping off (Camacho 2000). Seeds of the genus *Dodonaea* are impermeable (Cook *et al.* 2008), thus seed water uptake only occurs after mechanical or acid scarification or immersion of seeds in hot water (Camacho 2003; Baskin *et al.* 2004; Turner *et al.* 2009); complete germination in this species takes between 15 and 30 days (Camacho 2000; Benitez-Rodríguez 2005; Pedrero-López 2011). In species exhibiting physical dormancy, weakening of the seed coat or the aperture of a specialised 'water gap' may also occur in the soil through the effects of daily temperature fluctuations (Baskin & Baskin 2000) or exposure to heat shock during wildfires (Hodgkinson & Oxley 1990; Hodgkinson 1991). Some reports suggest that seed coats can also be weakened by fungal activity in both impermeable and permeable seeds in the soil (Pfeiffer 1934; Gogue & Emino 1979; Morpeth & Hall 2000; Sánchez-Coronado *et al.* 2011).

Dodonaea viscosa is a small tree that has been proposed as appropriate for the restoration of arid and rocky areas because of its tolerance to dryness and its high biomass production (*Dodonaea* litter improve soil quality). Additionally, this species has both medicinal and ornamental qualities; its branches are also used as supports in horticultural practices (Camacho 2000, 2003). Hence, we selected this species for application of a priming treatment on the seeds to improve germination rate (velocity), synchrony and seedling tolerance to an adverse environment (water and temperature stress).

We hypothesised that natural priming may improve seed and seedling vigour even in species with physical dormancy, once this has been overcome. Seed vigour is expressed as a higher germination rate (velocity) and synchrony and a shorter lag time; seedling vigour, among other factors, is expressed as higher survival. Both can have practical and economic advantages in restoration programmes: fast germination can shorten time in a shade house and the higher seedling survival might facilitate plant propagation. To apply natural priming (in the soil) to the physically dormant seeds of *D. viscosa* we first determined the minimum scarification time required to make the seeds sufficiently permeable for hydration and dehydration in the soil, but insufficient for the whole seed population to germinate during the natural priming treatment. For this purpose, seeds were exposed to different periods of acid scarification. Second, we determined whether duration in the soil modified the seed coat sufficiently to overcome physical dormancy in the whole seed population and would consequently favour priming of all the seeds in the soil. For this purpose, we evaluated changes in physical dormancy loss by examining seed permanence in the soil when all the buried seeds were pre-scarified or not, and were exposed to a post-scarification treatment. Third, we considered it relevant to know which microenvironment is adequate for seed burial. This was assessed through germination of seeds previously buried in three contrasting microenvironments inside the Parque Ecológico de la Ciudad de México (PECM, open, gap and forest sites). Fourth, we determined the effect of burial on seed germination and seedling survival, which are the main constraints for the use of *D. viscosa* in the restoration of harsh environments and for its propagation in nurseries. Fifth, we confirmed that seeds experience natural priming in the soil through analysis of protein mobilisation in the seeds.

Because it has been suggested that seeds of *D. viscosa* may be physiologically dormant, as evidenced by an unusually long lag time for germination, even after acid scarification (>1 month; Plata-Álvarez 2002), we also determined the effect of scarification, gibberellin and after-ripening on seeds of two different ages. Finally, we determined if the environment of the collection site (maternal effect) modifies the germination response to burial between seeds from two elevations inside the study area, as occurs in other species growing in the study area (*i.e. Buddleja cordata*; González-Zertuche *et al.* 2002).

MATERIAL AND METHODS

Study site

The study was carried out in two protected areas located in the southern portion of the México Valley: Parque Ecológico de la Ciudad de México (PECM, 19°15′32″N, 99°12′1.9″W, 2400–2850 m a.s.l.) and El Pedregal de San Ángel (REPSA, 19°18′21″ N, 99°12′4″W; 2292–2365 m a.s.l.). Because of the elevation, the climate is temperate, with a long sub-humid fresh summer, Cb′(w2)(w). Mean annual temperature at the lowest elevation is 14 °C, and at 3000 m, it is 11 °C; mean annual precipitation is 880 and 1100 mm, respectively; 80% of the precipitation occurs between June and October (Rzedowski & Rzedowski 2001; González-Hidalgo *et al.* 2002). Both protected areas are in lava fields approximately 1650–2000 years old (Siebe 2000); previous to the last eruption, oak forests were present. Evidence of oak forests remains in the deepest soils and in areas with low disturbance. The upwelling lava rock is covered with xerophylous shrubland. The presence of xeromorphy is a result of high rates of water percolation and decreased water retention of the shallow soils. The PECM presents different degrees of disturbance, from removal of vegetation and rock with machinery to selective extraction of wood for charcoal. The PECM and REPSA provide several ecosystem services to Mexico City, including, importantly, recharging of the city aquifers.

General procedures

Seeds were collected in the REPSA and PECM from at least 20 individuals per site from December 2002 to January 2003. In the laboratory, seeds were manually separated from fruits and

stored in glass containers under laboratory conditions (21.63 ± 1.8 °C, RH = 38.7%). In all cases, seeds were germinated at 25 °C in Petri dishes on agar plates (1% in water). In addition, the dishes were placed inside germination chambers (Lab-Line Instruments, Melrose Park, IL, USA) equipped with fluorescent cool white (F20T12/CW, Sylvania, 20 W) and incandescent (Solar, 25 W) lamps, with a photoperiod of 12 h/12 h (light/dark). Experimental designs included three or five replicates of 30 seeds per Petri dish per treatment, depending on seed availability. Over 30 (treatments) or 60 (controls) days, radicle protrusion (germination) was recorded every other day. Observations in controls were longest (60 days) in order of confirm germination of any further seeds.

Recently collected and 2.5-month-old seeds

To determine the minimum time to permeability of the seed population, we assessed the depth of physical dormancy and the presence of breaking of physiological dormancy promoted by gibberellin treatment. For recently collected seeds from the PECM, physiological dormancy was assessed by adding 0 or 500 ppm GA_3 (Sigma-Aldrich, St. Louis, MO, USA) to the agar. To test for physical dormancy, recently collected seeds (Control-1, untreated seeds) were scarified by immersion in sulphuric acid for 0, 2, 4, 6, 8 or 10 min (98% H_2SO_4; Baker, Mexico). Immediately after acid scarification, the seeds were washed with tap water for 2 min and then sown in Petri dishes. The experimental design consisted of two GA_3 concentrations (0 and 500 ppm) × six scarification times (0, 2, 4, 6, 8 or 10 min), *i.e.* 12 treatments. Similar scarification and gibberellin treatments were applied to seeds stored under laboratory conditions for 2.5 months (Control-2, untreated seeds). In this case we used two GA_3 concentrations (0 and 500 ppm) × four scarification times (0, 2, 6, or 10 min), *i.e.* eight treatments. Results for these scarification times were compared with those obtained for recently collected seeds.

Non-pre-scarified and pre-scarified seeds buried and collected in the PECM

From the previous treatments, a minimum pre-scarification time of 3 min was established for all the pre-scarified seeds that were buried. This period may favour changes in seed hydration in the soil but does not favour germination during burial. Pre-scarified (3 min) and non-pre-scarified seeds were buried at a depth of 5 cm in the PECM soil in three environments: open, gap and forest sites. The seeds were unearthed 2.5 months after burial, dried in darkness in laboratory conditions (16–24 °C, RH 35–50%). Because prior to burial the pre-scarification time was insufficient to totally break physical dormancy, after burial the seeds were post-scarified through immersion in sulphuric acid for 0, 2, 6 or 10 min (post-scarification) and then germinated.

Seeds collected and buried in the PECM or in REPSA and buried in the site of collection

To test whether the environment during seed development and germination modifies physical dormancy, seeds collected in the REPSA and PECM were divided into three independent groups at the same time as in the previous treatments. One group from each seed collection was buried in an open site, another under the shade of *Buddleja cordata* trees (shaded site) and the final group in a volcanic rock crack where soil had accumulated. Seeds were immediately buried or after 2.5 months and unearthed as in the previous treatment, dried then germinated along with controls. Unearthed seeds and control seeds were scarified for 3 min or not scarified, respectively. The experimental design consisted of two seed origins × three seed burial sites × two scarification treatments, *i.e.* 12 treatments. Seeds were germinated in the laboratory at 25 °C.

Seedling survival in the PECM

In June 2003, PECM Control-2 seeds and pre-scarified seeds (those unearthed from the open site), with 250 seeds per treatment, were post-scarified for 10 min to ensure high germination. Seeds were sown in black plastic bags (4 × 25 × 12 cm) containing 1 l of forest soil, with one seed per bag, and watered to field capacity every other day. In August 2003, 80 surviving seedlings from each treatment, at 3 months old, were transplanted to an abandoned site 2-m wide in the xerophylous shrubland, which was covered by perennial shrubs and deciduous grasses and annual herbs. Survival percentages were then followed from September 2003 to September 2004.

Protein patterns of control, unearthed and germinated seeds

Laboratory-stored seeds, non-pre-scarified unearthed seeds, pre-scarified unearthed seeds and germinated seeds buried in the open site were used. The seed storage protein-enriched fractions, which contain globulins and vicilins, were obtained as previously described (Gamboa-deBuen *et al.* 2006) following the instruction manual of the BD Phosphoprotein Enrichment Kit (Clontech, Mountain View, CA, USA). The protein samples (50 μg) were run on a 12% SDS-PAGE gel and stained with Coomassie blue. To characterise the seed structure and protein locations, we conducted a histochemical analysis using the Naphthol blue technique (López-Curto *et al.* 2005).

Data analysis

Control-1, the control for the recently collected seeds, was compared with Control-2, the control for all the seeds 2.5 months old either buried or laboratory-stored. Cumulative germination percentages were arc-sin transformed and fitted to the best model, the exponential sigmoid curve using Table Curve 2D version 3 (AISN Software, Chicago, IL, USA):

$$y = a/[1 + b^{(-cx)}]$$

All the fitted curves had $R^2 \geq 0.96$ and $P \leq 0.00001$. Germination rate was determined as the first derivative maximum of the exponential sigmoid curve, and lag time was calculated from the generated curve. Effects of the experimental factors on final germination percentages (arc-sin transformed) were tested with multi-factor analysis of variance, and multiple comparisons were performed using Tukey tests from Statgraphics version 5.0 (Statistical Graphics, Englewood Cliffs, NJ, USA). In all cases, normality and homoscedasticity assumptions were fulfilled; these tests were also done with Statgraphics. All the

analyses were performed with germination data obtained 30 days after sowing.

RESULTS

Recently collected and 2.5-month-old seeds

In the group of recently collected seeds, percentage germination increased significantly with scarification time ($F_{(5,35)} = 209.78$, $P = 0.00001$). Seeds lacking scarification treatment had little, if any, germination (0.66 ± 1.19). Seed germination (>80%) was increased after scarifying the seeds for 6, 8 or 10 min, without any significant difference observed among scarification periods. Seeds scarified for 2 min had 50% germination, while those scarified for 4 min had 76.55% germination, with no significant difference from the highest germination percentages obtained after scarification for 6–10 min (Fig. 1A). Gibberellin treatment had no significant effect on germination ($F_{(1,35)} = 0.11$, $P = 0.74$). Similar results were found in 2.5-month-old seeds; however, in these seeds, gibberellin had a significant inhibitory effect on seeds scarified for 6 and 10 min ($F_{(3,39)} = 10.71$, $P = 0.00001$; Fig 1B). The GA_3 alone did not have any significant effect, but germination was increased after scarification ($F_{(3,39)} = 101.7$, $P = 00001$); with 10 min scarification leading to the highest percentage germination ($F_{(3,39)} = 391.08$, $P = 0.00001$). In comparison to recently collected seeds, seeds stored in the laboratory for 2.5 months had significantly lower percentage germination ($F_{(1,\ 63)} = 121.81$, $P = 0.00001$). Seeds scarified for 10 min had similar percentage germination to those scarified for 6 min in recently collected seeds (Fig. 1A, B). In addition, effects of the interactions of seed age with scarification time ($F_{(3,\ 63)} = 58.47$, $P = 0.00001$), scarification time with gibberellin treatment ($F_{(3,\ 63)} = 5.49$, $P = 0.002$) and the triple interaction ($F_{(3,\ 63)} = 2.97$, $P = 0.04$) were significant (Fig. 1A, B).

Non-pre-scarified and pre-scarified seeds buried and collected in the PECM

Pre-scarification, burial site and time of post-scarification were factors that significantly affected germination ($F_{(1,239)} = 144.25$, $P = 0.00001$; $F_{(2,239)} = 3.57$, $P = 0.03$; $F_{(3,239)} = 456.1$, $P = 0.00001$, respectively). Interactions between pre-scarification and burial site ($F_{(6,239)} = 4.38$, $P = 0.01$), pre-scarification and time of post-scarification ($F_{(3,239)} = 21.13$, $P = 0.00001$) and time of post-scarification with burial site ($F_{(6,239)} = 24.68$, $P = 0.00001$) also affected germination. Moreover, the triple interaction was significant ($F_{(6,239)} = 2.33$, $P = 0.03$). The success of post-scarification treatment on seed germination was dependent of the microsite where the seeds were buried, the pre-scarification treatment and the duration of post-scarification treatment to which the seeds were submitted (Fig. 2). After unearthing seeds from all the burial sites, pre-scarification was found to significantly reduce the differences between germination obtained with post-scarification times and germination of the seeds buried without pre-scarification. In addition, germination of the pre-scarified seeds, even without post-scarification treatment, increased percentage germination (27–47%; Fig. 2D–F) compared to those that were not pre-scarified (4% compared to 22%; Fig. 2A–C).

Germination rate differed among treatments, and post-scarification time had the most effect ($F_{(3,71)} = 2133.25$, $P = 0.00001$), followed by the effect of burial site ($F_{(2,71)} = 2037.82$, $P = 0.00001$) and the effect of gibberellin treatment ($F_{(1,71)} = 694.95$, $P = 0.00001$). All the interactions were also significant ($F > 424.95$, $P = 0.00001$ for all interactions). The germination rate was significantly higher in seeds pre-scarified for 10 min, buried in the gap site, and germinated without gibberellin treatment. The slowest response was for the seeds pre-scarified for 6 min, buried in the open site and germinated with gibberellin treatment. Non-scarified seeds germinated faster than these due to very low percentage germination, and these few seeds generally germinated rapidly.

There were no significant differences in the lag time between treatments, which was ~2 days.

Seeds collected and buried in the PECM or REPSA and buried in the site of collection

In both seed populations and in control and unearthed seeds, scarification significantly increased percentage germination ($F_{(1,47)} = 4636.15$, $P = 0.00001$; Fig. 3). Scarified control seeds from both populations had similar percentage germination. However, after burial, the PECM seeds had lower percentage germination than the REPSA seeds ($F_{(1,47)} = 48.23$, $P = 0.00001$). Percentage germination of seeds unearthed from the shade site and scarified was slightly significantly higher than in the two other burial sites, independent of the seed population and in comparison to the controls ($F_{(3,47)} = 35.22$, $P = 0.00001$). In the PECM, seeds that were unearthed from open and shaded sites and that were not scarified had significant and slightly higher percentage germination than control seeds and those buried in rock crevices ($F_{(3,47)} = 22.43$, $P = 0.00001$).

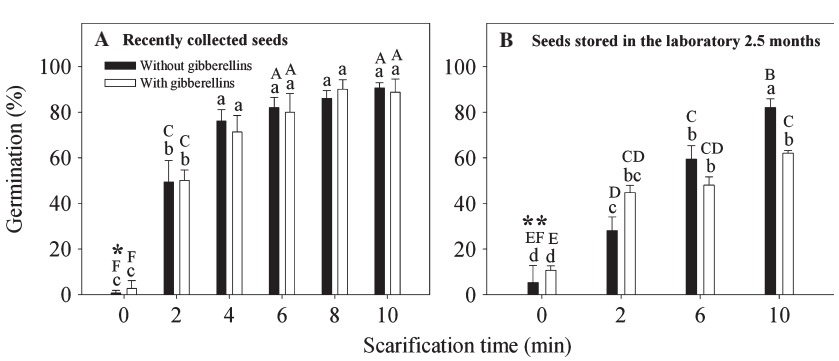

Fig. 1. Effects of gibberellin and acid scarification on the germination of recently collected A: and 2.5-month-old B: seeds of *Dodonaea viscosa*. Bars represent the mean ± SD (n = 5 replicates), and different letters indicate significant differences at $P < 0.05$. Comparisons were done inside each panel. *Control-1, **Control-2.

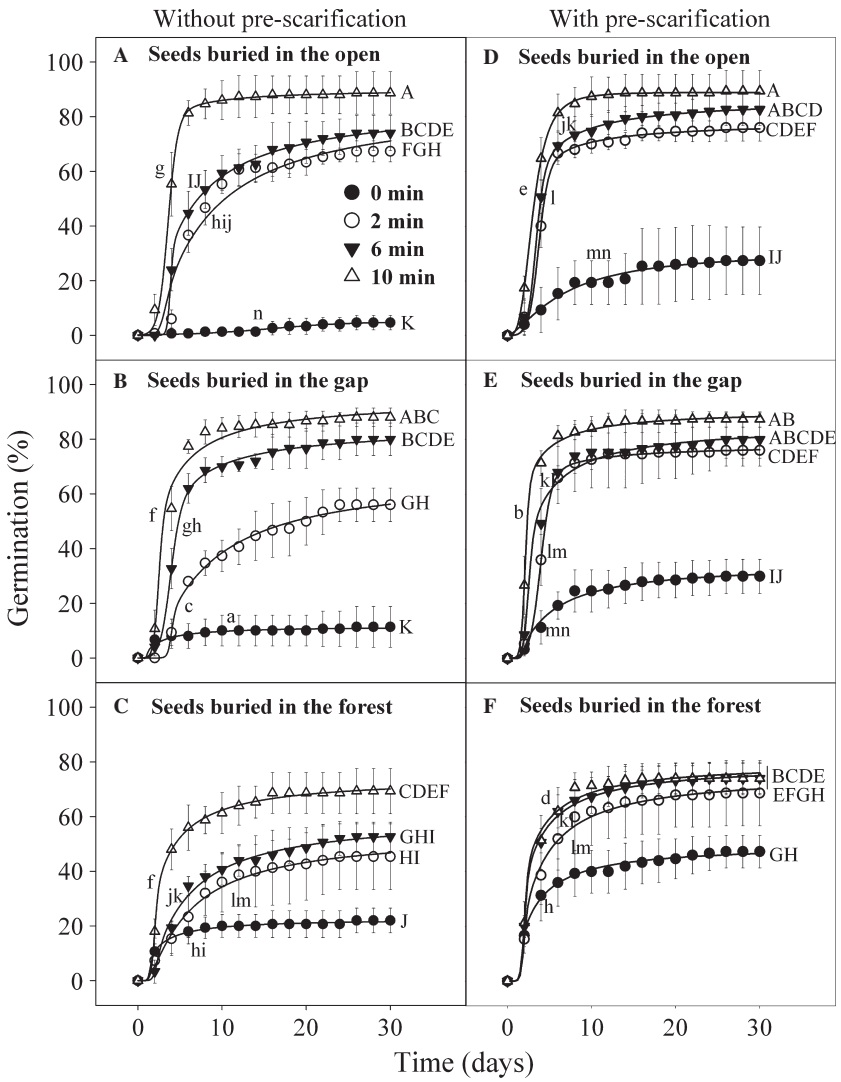

Fig. 2. Cumulative germination of *Dodonaea viscosa* seeds following burial in three microsites in the Parque Ecológico de la Ciudad de México. Prior to burial, half of the seeds were pre-scarified for 3 min. After the seeds were unearthed, both groups of seeds were submitted to the indicated post-scarification treatments. Bars represent the mean ± SD (n = 5 replicates). Small letters indicate significant differences between germination rates and capital letters indicate significant differences between germination percentages at $P < 0.05$ in all the cases.

Seedling survival in the PECM

A total of 98% of seedlings from unearthed seeds survived during their stay in the shade house, while only 32% of seedlings of control seeds survived. There was a similar pattern in the seedlings transplanted to the field, where 50 ± 4% of seedlings of unearthed seeds survived, while 32 ± 4% of seedlings from control seeds survived.

Protein patterns in control, unearthed and germinated seeds

Protein patterns from the enriched fractions of seed storage proteins are provided in Fig. 4. A 48-kDa protein was found in extracts from control seeds and in non-pre-scarified seeds that were unearthed from the open site, but not in the seeds pre-scarified before burial. This protein band was also absent in protein extracts from germinated control seeds. Transverse sections of seeds had long spirally coiled cotyledons and protein bodies distributed throughout these structures and along the embryonic axis. The cotyledons are the primary storage tissue, and the 48-kDa protein was found inside protein bodies in these organs (Fig. 5).

DISCUSSION

After collection, seeds of *D. viscosa* did not exhibit physiological dormancy, unlike reports for seeds collected in Mexico (Camacho 2000) and in other localities, including Hawaii and New Zealand (Baskin *et al.* 2004). This was demonstrated as similar germination between seeds treated or not treated with gibberellin and the high percentage germination of seeds scarified for 10 min. Even after more than 2.5 months of laboratory storage, gibberellin had a slight but significant inhibitory effect on seed germination. This effect was observed when the seeds were scarified for 6 and 10 min. In seeds scarified for 2 min, gibberellin improved seed germination, but not significantly. Thus, the contrasting germination responses to gibberellin can be linked to seed age and scarification treatment. In addition, the depth of physical dormancy can vary during storage, especially if seed coats become more permeable during storage, as reported previously (Jayasuriya *et al.* 2008). In some cases, gibberellin treatment can be toxic to the embryo (Olvera Carrillo *et al.* 2003). The extent of physical dormancy was deep: seeds required at least 6 min of immersion in H_2SO_4 to remove dormancy. In most of the seed population, the longest period

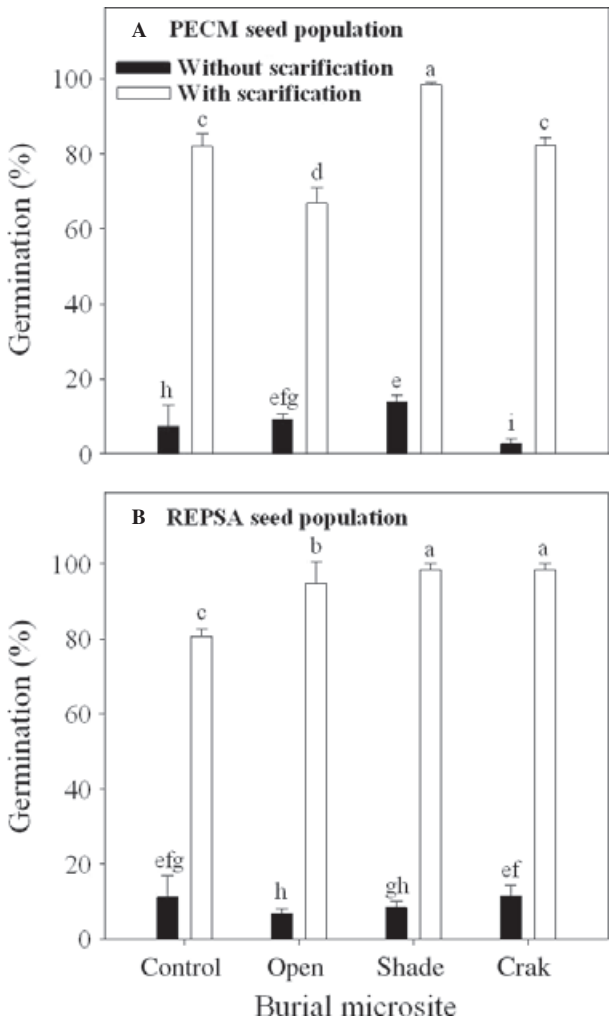

Fig. 3. Germination of two *Dodonaea viscosa* seed populations collected in the Parque Ecológico de la Ciudad de México (PECM) and in the Reserva Ecológica del Pedregal de San Ángel (REPSA) soil of three microsites, in their respective collection site. After 2.5 months buried, the seeds were unearthed and submitted to 3 min of scarification. Bars represent the mean ± SD (n = 5 replicates), and different letters indicate significant differences at $P < 0.05$.

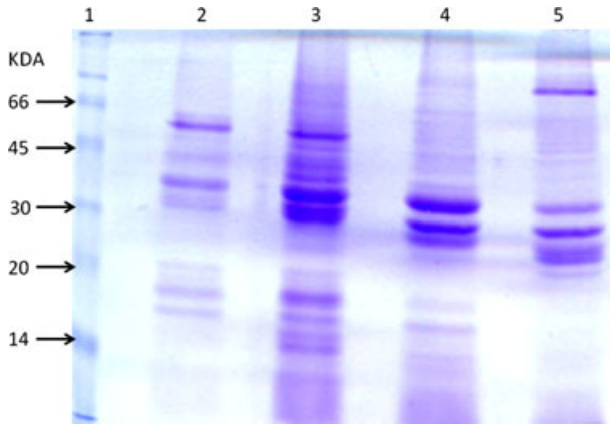

Fig. 4. Effects of seed hydration on the seed storage protein-enriched pattern. Marker (1), control seeds (2), non-pre-scarified exhumed seeds (3), pre-scarified exhumed seeds (4), control germinated seeds (5). A 12% SDS-PAGE gel was used and equal amounts (50 μg) of protein were loaded. The gel is representative of three replicates from three different seed batches.

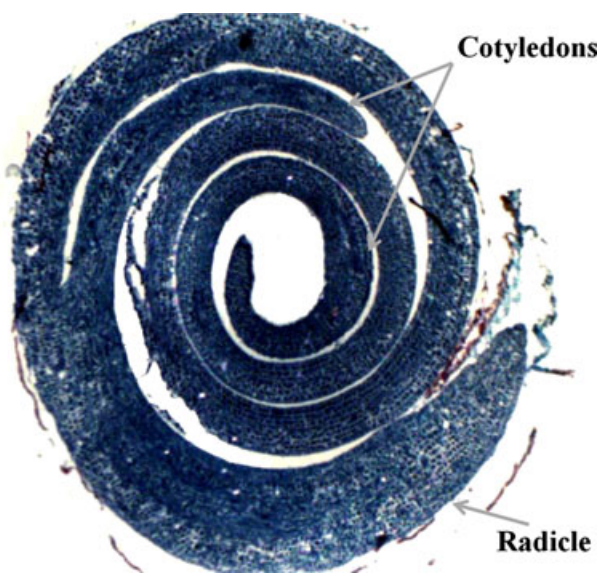

Fig. 5. Transverse section of a *Dodonaea viscosa* dry seed. Conspicuous protein bodies (stained of blue) are present in the embryo.

(10 min) of exposure to H_2SO_4 did not damage the seeds. Immersion of seeds in hot water produces 60% (2.5 min in hot water) or 34% (5 min) germination (Baskin *et al.* 2004; Pedrero-López 2011), which is lower than the germination percentages obtained following acid scarification in the present study (90.66%). However, seed germination also varies widely between seed lots, as observed after immersion of seeds at 75 °C for 3–8 min and the resulting 50–98% germination success (Camacho 2000). In these papers, time to reach full germination was between 15 and 30 days. In the present research this time was reduced to 5 days mainly in seeds buried in the forest, even without seed post-scarification.

Physical dormancy in the genus *Dodonaea* is related to the presence of a specialised 'water gap' in the seeds, which can be opened with high temperatures in hot water or in moist sand at 20–35 °C (Turner *et al.* 2009). Midday temperatures in the REPSA and in PECM can reach 60 °C (Gamboa-deBuen *et al.* 2006; Olvera-Carrillo *et al.* 2009a; respectively). In spite of these cues, seeds of *D. viscosa* remained dormant for ~75 days in the three sites of the lava field and exposed to sporadic precipitation; physical dormancy was not broken in non-pre-scarified seeds. After 2.5 months in the laboratory, only 11.2% and 7.48% of the seeds germinated (REPSA and PECM seeds, respectively); unearthed seeds from these two populations had slightly increased germination. Previously, it was reported that of *D. viscosa* seeds sown in the REPSA soil surface for 232 days, 32% germinated if they were maintained in a site shaded by *B. cordata* trees, while seeds that spent that time in crevices in rocks or in an open site had germination of 21% and 16%, respectively (Plata-Álvarez 2002). These data suggest that despite the wide temperature fluctuations that are common to the dry season and at the beginning of the rainy season in lava fields (Mendoza-Hernández *et al.* 2013b), *D. viscosa* maintains deep physical

dormancy in a large percentage of the seed population. As a consequence, this species may form a permanent seed bank, with different fractions of the seed population germinating under different circumstances and in different years.

Seeds with physical dormancy only take up water through 'water gaps' (also called 'strophioles' or 'lens', among others; Baskin 2003); however, in this study, all seed coats were weakened by H_2SO_4, which favoured seed water uptake and germination. During chemical scarification, the temperature only increased to 26 °C, which may not open the *D. viscosa* 'water gap' as effectively as hot water (Turner *et al.* 2009). This hypothesis could be tested using observations from scanning electron micrographs.

Seeds of *D. viscosa* do not require light for germination (Plata-Álvarez 2002); nevertheless, seed germination did not occur during burial, suggesting that additional soil factors, such as gases in the soil environment (*e.g.* CO_2, O_2; Karssen 1980/1981) might inhibit germination during this time. The effects of burial depended on the pre-scarification treatment. However, in the unearthed seeds, pre-scarification enhanced percentage germination with respect to the Control-2 seeds, independent of the burial site. Burying *D. viscosa* seeds in forest soil induced up to 47% and 22% germination in unearthed pre-scarified and non-pre-scarified seeds, respectively. This observation may be related to consistently higher moisture levels in the deep forest soil, as opposed to shallow soils in the open site and in rock crevices, which also lack a continuous canopy during the dry season that buffers daily temperature fluctuations. This differential effect of the burial site on seed germination has also been observed in other species, such as *Buddleja cordata* (González-Zertuche *et al.* 2000) and *Wigandia urens* (González-Zertuche *et al.* 2001; Gamboa-deBuen *et al.* 2006), which also grow in the PECM. In permeable but hard-coated seeds of *Opuntia tomentosa*, it has been reported that soil moisture in shaded areas favours fungal development, which weakens the seed cover thus increasing seed water uptake (Olvera-Carrillo *et al.* 2009b; Sánchez-Coronado *et al.* 2011).

Germination of the unearthed, non-pre-scarified seeds was enhanced by scarification time, *i.e.* germination increased as scarification time increased. In seeds pre-scarified and buried, 2 min of post-scarification was as efficient as 10 min in promoting high percentage germination. The effects of pre-scarification on seed water uptake during burial were also reflected in protein expression patterns. In pre-scarified seeds, which were visibly hydrated (increased in size) immediately after unearthing, the 48-kDa protein band observed in control seeds and in non-pre-scarified unearthed seeds was not detected. The 48-kDa protein band might be a seed storage protein that is mobilised during natural priming, as previously described for *Wigandia urens* seeds, which undergo natural priming (hydration–dehydration cycles) in the soil (Gamboa-deBuen *et al.* 2006). As in the laboratory priming treatments (Rajjou *et al.* 2012), the benefit of natural priming was maintained after seed dehydration. Seeds of *D. viscosa* had a large number of protein storage bodies in the cotyledons and in the embryonic axis. It is common for proteins contained in the axis to be used early during the germination process (Bewley & Black 1994). This fact should be studied for *D. viscosa*.

Our results also demonstrated that the effects of permanence of seeds in the soil depend on the degree of seed permeability, which may permit water uptake during burial. As a result, seeds may undergo natural priming, as documented in our study in the high percentage survival of seedlings of primed seeds, in contrast to those from Control-2, in both the shade house and in the field, and in the extent of protein mobilisation. These parameters were more important than germination parameters, including germination rate (velocity). As we might expect of the natural priming effect, germination rate, synchrony and time to complete germination were significantly improved in primed seeds (see Fig. 2), but in this study were mainly determined or masked by the scarification treatments. However, the effect of burial alone is evidenced through the differences in germination between the three burial sites.

In other species, the tolerance of seedlings to stress has been attributed to the synthesis of heat shock proteins of 14–23 kDa (González-Zertuche *et al.* 2001; Chen & Arora 2012). However, in *Dodonaea* species, we did not study the fraction of thermostable proteins. Thus, further research should be performed to understand the relevance of natural priming in each step of the seed's life and the consequences for seedling performance, which may improve germination and seedling success in restoration programmes based on the use of native plants. Seed burial has proven to be a potentially inexpensive technique that may improve germination and seedling establishment in *D. viscosa*, and could be tested as a propagation technique for other species.

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