

Research Paper

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Priming effects on seed germination of two perennial herb species in a disturbed lava field in central Mexico

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Abstract

Priming has proved to enhance seed germination, but most of the research dealing with this topic has been carried out with cultivated species. The potential applications that this process has on wild species, which can be useful for restoration, are usually overlooked. This study analyses the germination response after natural priming and hydropriming of *Penstemon roseus* and *Castilleja tenuiflora*, two perennial herbs growing in a protected area known as 'Parque Ecológico de la Ciudad de México'. Photoblastism was evaluated for both species. Seeds were exposed to a hydration/dehydration cycle and then placed in germination chambers to determine responses to hydropriming. To identify the effects of natural priming, seeds were buried in natural conditions and then recovered every two months and placed in germination chambers. Germination percentages and rates were then quantified. Both species proved to have permeable seed coats. *Penstemon roseus* seeds are positive photoblastic whereas *C. tenuiflora* seeds are indifferent to light. Priming methods increased *C. tenuiflora* germination rates, but they did not affect germination capacity. For *P. roseus*, priming methods did not improve germination rates, and germination capacity of recovered seeds decreased after the rainy season, suggesting that *P. roseus* forms a short-term, transient, seed bank. The germination strategies of these two species allow them to occupy suitable microsites for germination and establishment. These responses can be helpful in developing restoration programmes based on the accelerated establishment of native and characteristic successional species.

Introduction

Urban development leads to loss of the natural areas surrounding cities. This results in reduced provision of valuable ecosystem services such as water filtration and groundwater recharge (Vitousek, 2006). Urban expansion has also been shown to cause fragmentation and reduction of the habitats of native species, replacing them with widespread exotics, including invasive species (McKinney, 2002). Ecological restoration of these areas should therefore focus on the re-establishment of native vegetation and its biotic interactions.

In a disturbed environment, perturbation can modify the germination and establishment conditions of some species; for example, light exposure can trigger germination in seeds buried in rock or soil fissures. Thus, disturbance may induce germination in many positive photoblastic species that require light to germinate (Wesson and Wareing, 1969).

Germination is a complex process that is regulated in many ways (Bewley and Black, 1994). It influences species recruitment and seedling behaviour over time; for example, species that are recruited early tend to pre-empt resources. From that point, germination affects the dynamics of plant populations as well as the structure and composition of communities (Silvertown *et al.*, 2005).

Various methods, such as seed priming, have been utilized in order to improve the germination process. Priming activates the metabolic changes associated with germination, which then halt prior to radicle protrusion. When the seeds are rehydrated, the physiological changes associated with the germination process are re-assumed, and the seeds then germinate more promptly (González-Zertuche *et al.*, 2000). Priming may result in increased germination rates (velocity), final percentages and uniformity and can also improve seedling emergence and establishment under stressful conditions (Bray, 1995; Halmer, 2004).

Hydropriming consists of hydration of seeds, thereby triggering the metabolic functions associated with germination. After soaking, the seeds are dried to prevent germination and then rehydrated, with the benefits described above (Bray, 1995). It has been shown that seeds buried in the soil have a higher chance of undergoing natural priming mediated by

fluctuations in their environmental cues (González-Zertuche *et al.*, 2001). Natural priming can take place in buried seeds, enhancing several germination parameters, and can produce results like those found in experimental priming treatments (González-Zertuche *et al.*, 2001). Consequently, seeds that have undergone natural priming can germinate faster than those that did not undergo this process, making the use of space, water and nutrients more efficient for these seedlings.

The benefits of priming have been reported widely in different crop species; however, little is known about the effects of priming on wild species. Priming can influence the dynamics of populations and communities and has potential applications through the use of native species of value for ecological restoration and reforestation of disturbed habitats (González-Zertuche *et al.*, 2000).

The 'Parque Ecológico de la Ciudad de Mexico' (PECM) is a natural area embedded in Mexico City and contains several vegetation types, including different temperate forests and a shrubland area established in a large lava field. This natural area was invaded by illegal urban settlements from 1980 to 1989 and this action disturbed part of the xerophyllous shrubland and the oak forest that characterize the vegetation of the PECM (Cano-Santana *et al.*, 2006; Pedrero-López *et al.*, 2016). In response to this invasion, different strategies were adopted to recover the disturbed areas. One of the strategies involved the creation of a restoration programme, part of which has involved different studies of the germination of native species (Soberón *et al.*, 1991; González-Zertuche *et al.*, 2002; Martínez-Villegas *et al.*, 2012; Mendoza-Hernández *et al.*, 2013).

In this study, we analysed whether there are effects of natural priming and hydropriming on the germination of *Castilleja tenuiflora* (Orobanchaceae) and *Penstemon roseus* (Plantaginaceae), two characteristic species of the PECM zones where the shrubland has been disturbed but not completely destroyed. In addition, germination responses to light were evaluated for both species. Given that both natural priming and hydropriming trigger the germination metabolic process in advance, a higher germination response in a shorter time is expected in seeds that have undergone priming. The small size of the seeds and the seasonal environment in which these two species grow suggest a positive photoblastic response in both species (Vázquez-Yanes and Orozco-Segovia, 1990). By studying the photoblastic response and the effect that priming has on these two wild non-exotic species, we will be able to understand some of the physiological aspects that confer ecological advantages to these colonizing herbs. This could be of value for the identification of methodologies with potential applications in restoration strategies.

Materials and methods

Species and study area

Castilleja tenuiflora Benth

(Orobanchaceae) is a perennial erect herb with a very ramified stem. Flowers are present throughout the year, but are more abundant from late summer to early autumn; fruits are produced from late autumn to early winter. Seeds are small (1.8 × 0.8 mm) and covered with a reticular seed coat. The species is widespread in temperate forests, shrublands, grasslands, crop edges and roads (Rzedowski and Rzedowski, 2005). It has been reported as a root hemiparasite, e.g. of *Lupinus montanus* (Heckard, 1962; Montes-Hernández *et al.*, 2015); however, its potential interaction with other host plants remains unknown.

Penstemon roseus (Cerv. ex Sweet)

(Plantaginaceae) is also an erect perennial herb, widely distributed in the Basin of Mexico on mountain slopes, oak and oak-pine forests, shrublands and disturbed areas (Rzedowski and Rzedowski, 2005). Flowers are produced from late summer to early autumn, and fruits from autumn to winter. Seeds are dark brown and small (1.8 × 1.2 mm) and are irregular in shape with a rugged coat (Martínez-Orea *et al.*, 2012).

Study site

The study area was the 'Parque Ecológico de la Ciudad de México', a protected natural area of importance because of its contribution to recharging the aquifer of the Basin of Mexico and other environmental services (Cano-Santana *et al.*, 2006). It has an area of 720 ha and is located in the southern part of the Basin of Mexico on Mt Ajusco, at 2650–2800 m above sea level. The climate is temperate with summer rains; mean annual precipitation is 1000 mm and mean annual temperature is between 12 and 18°C with a maximum of 31°C and a minimum of -5°C (Soberón *et al.*, 1991). The PECM is volcanic in origin. The lava flow has created a complex system of patches of vegetation that include oak and oak-pine forests with well-developed soils, alternating with xerophyllous shrublands on the younger basaltic substrate. The basaltic fragmented rock presents low water retention and high evaporation rates that contribute to an edaphic condition of aridity. This condition makes seed germination and plant recruitment difficult (Martínez-Villegas *et al.*, 2012; Mendoza-Hernández, *et al.*, 2013).

General procedures

Fruits of *Castilleja tenuiflora* and *Penstemon roseus* were randomly collected during the late autumn and early winter of 2012, in the disturbed shrubland of PECM. Seeds were taken directly from the fruits and stored under dry conditions prior to the germination trials.

For the priming treatments, seeds of *P. roseus* were washed for up to 10 min in Tween 80 polyoxyethylene sorbitan monolaurate (Lipoquimia, Mexico) (three drops in 50 ml of distilled water) and then soaked for 20 min in Microdyn (colloidal silver solution at 35%, Mercancías Salubres S.A. de C.V., Mexico: three drops in 50 ml of distilled water). Finally, seeds were disinfected in 70% ethanol and washed in 50 ml of sodium hypochlorite solution (1:3 v/v) for 20 min (López-Escamilla *et al.*, 2000).

The *C. tenuiflora* seeds were disinfected only with 0.2% fungicide solution (Captan 50 [cis-N-[(trichloromethyl)thio]-4-cyclohexane-1,2-dicarboximide], AGM, Mexico) due to the loss of viability, determined with tetrazolium chloride after applying the disinfection process described above. The viability of *P. roseus* seeds could not be determined in this way, since the size and colour of the seeds did not permit observation of the tint in them. Seed viability was tested in 100 seeds of each species using X-rays (Ultrafocus Digital Radiography System, Tucson, AZ, USA). Fifty per cent of the seeds of *P. roseus*, and 60% of the seeds of *C. tenuiflora*, proved to be unviable.

Seeds were germinated on agar (2%) in 60-mm Petri dishes. For the hydropriming and control group, 20 seeds were sown in each Petri dish with a total of 15 replicates per treatment. Sample size for the natural priming experiments explained below depended on the number of seeds that were recovered every 2 months. All germination trials were carried out in a

germination chamber (54 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 25°C, Lab-Line Instruments, Inc., Melrose Park, IL, USA; fluorescent white light Osram Universal, 20 W, Brazil). Germination temperature was based on germination studies of other species from the PECM (González-Zertuche *et al.*, 2001, 2002; Mendoza-Hernández *et al.*, 2013).

Priming treatments

Natural priming

For natural priming, groups of 100 seeds of each species were placed in one of 18 pots made of cloth (10 cm width and 10 cm length) and filled with soil from the study area. Seeds were placed on the soil surface and covered with a light layer of soil. The 18 pots for each species were then randomly placed at different points of the disturbed xerophyllous shrubland in February 2013. Over the course of one year, three pots for each species were selected randomly and the seeds placed in them were recovered every 2 months. A total of five bi-monthly periods were evaluated. Germinated seeds were quantified and separated, while those that did not germinate were recovered and germinated in the controlled environment chamber, as explained above. Germination was monitored daily until completion.

Hydropriming

To test the effect of hydropriming, a portion of the collected seeds were soaked in the laboratory with an unlimited amount of distilled water for 24 h. The seeds were then dehydrated for one week by placing them between two pieces of absorbent paper towel wrapped with aluminium foil to prevent germination. Once dried, 300 seeds of each species were placed in the controlled environment chamber (20 seeds for each of the 15 Petri dishes) and their germination monitored daily until completion. Seeds without hydropriming treatment (serving as a control group) were germinated simultaneously in the chamber. Maximum germination rates, evaluated as the first derivative of the Hill Model (El-Kassaby *et al.*, 2008), and the probabilities of germination over time were determined for both species.

Light treatments

The photoblastic response of recently collected seeds was determined in five groups of 20 seeds of each species. Each group was exposed to white (R:FR = 4, PPD = 33.21 $\mu\text{mol m}^{-2} \text{s}^{-1}$), red (R:FR = 3.39, PPD = 5.18 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and far-red light (R:FR = 0.05, PPD = 0.12 $\mu\text{mol m}^{-2} \text{s}^{-1}$), as well as darkness. Seeds were sown in Petri dishes with absorbent paper inside plexiglass (Red no. 245; Blue no. 850, Rohm and Hass, Mexico) germination boxes (length 44 cm, width 34 cm, height 10 cm) to create the necessary light conditions. In a green light chamber (Green Screen L107, Plastimundo, México, with fluorescent white light lamps, Philips, 32 W), the boxes were opened to water the seeds when necessary. Maximum germination percentages were determined for each light treatment. No disinfection methods were used before these germination trials to avoid phytochrome triggering prior to light exposure treatments.

Germination models and statistical analysis

Final germination proportions for photoblastic response were analysed with a logit-regression model. For the priming experiment, a non-linear regression was used. The mean function fitted

was a Hill 4-parameter model (El-Kassaby *et al.*, 2008):

$$y = y_0 + \frac{y_{\max}x^\alpha}{C_{50}^\alpha + x^\alpha} \quad (1)$$

where the parameter y represents the cumulative germination probabilities at time x (days), y_0 is the intercept, y_{\max} is the germination capacity or the maximum cumulative germination probability, and α is the parameter that controls the shape and steepness of the curve: the higher the value of this parameter, the faster the asymptote of y_{\max} is reached. The value C_{50} is the time (in days) required for 50% of the viable seeds to germinate. The parameters of the models were estimated for each treatment with non-linear least squares (NLS) function implemented in R (R Core Team, 2016). Parametric bootstrap confidence intervals were calculated for the hydropriming, control and first natural priming period in which the results proved to be the best. The parameters were then compared with a one-way ANOVA and Tukey's HSD for all pairwise comparisons. In addition, the mean time of maximum germination rate ($TMGR$) was estimated according to El-Kassaby *et al.* (2008) as:

$$TMGR = \sqrt{\frac{C_{50}^\alpha(\alpha - 1)}{\alpha + 1}} \quad (2)$$

where $TMGR$ is the time required to reach the maximum instantaneous germination rate function, which is the first derivative of the mean function.

The times required to reach 10% of germination and dormancy index (DI) were also estimated. This index (Eqn 3), proposed by Richter and Switzer (1982), quantifies the increase in germination response by calculating the difference between the areas under germination curves with and without germination pre-treatment over time; i.e. a small difference signifies that the area under the curves is similar:

$$DI = \int_{t_0}^{t_n} (y_1 - y_2)dt \quad (3)$$

To assess the response in the early, middle and late steps of the germination process, we computed the dormancy index restricted to 30, 15, 10 and 5 days. If $DI \leq 0$ the treatment did not improve the germination response, and non-parametric bootstrap confidence intervals were calculated to prove the significant difference. If 0 was contained within the interval, then the differences were not significant (Fig. 1). Parameter y_0 , which is related to basal response, was excluded since it is assumed that the germination response had not yet begun at time zero. In order to prove the hypothesis that $y_0 = 0$, F -tests were conducted following Ritz and Streibig (2008), and to test the effect of natural priming during the five bi-monthly periods, parameters of the models were compared with a Kruskal-Wallis test, since departures from normality were evident in the qqplot graphs. A Fligner-Killeen test was used to verify the assumptions of homoscedasticity and a Dunn's test was performed to test for significant differences between treatments.

Software

Exploratory analysis, variable selection and modelling steps were written in the open source statistical package R, version 3.1.3

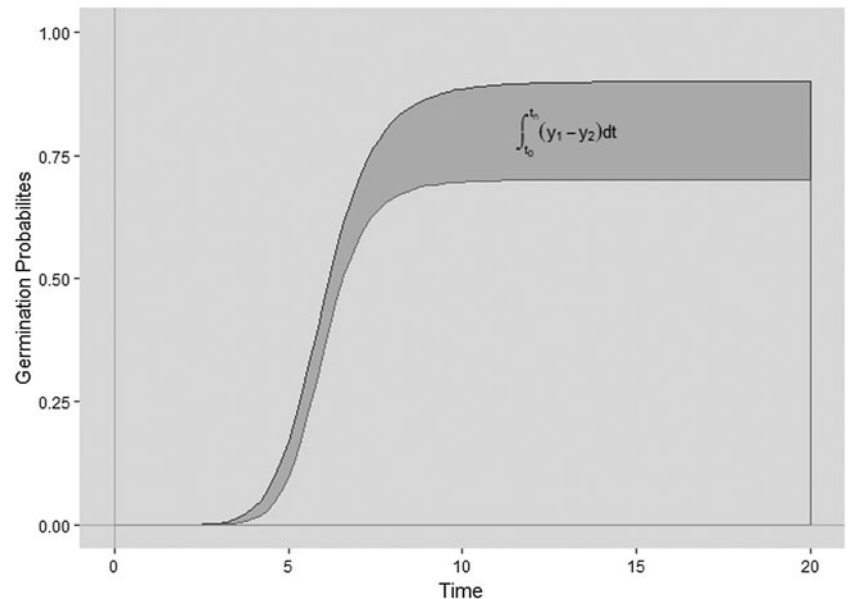


Figure 1. Dormancy index from t_0 to t_n for two treatments: y_1 ($\alpha = 8$, $C_{50} = 6$, $y_{\max} = 0.9$) and y_2 ($\alpha = 8$, $C_{50} = 12$, $y_{\max} = 0.7$).

(R Core Team, 2016). For the normality tests, the Nortest package was used. The package PMCMR was used for the non-parametric *post hoc* tests.

Results

Photoblastic response

Seeds of *C. tenuiflora* germinated under all of the light treatments including darkness, although germination did not exceed 0.68 in any case. In darkness, the probability of germination was significantly lower than in the different light treatments ($D = 25.37$, $P < 0.05$) (Fig. 2a). Seeds of *P. roseus* had low probabilities of germination in red and white light, ~ 0 in FR, and presented no germination in darkness (Fig. 2b).

Germination curves and priming treatments

The cumulative probabilities of germination of *C. tenuiflora* and *P. roseus* in control seeds and those treated with hydropriming or natural priming, and the *quasiR*² values suggest that the Hill 3-parameter model is correctly specified as a mean function for both species.

There were no significant differences among treatments for the maximum cumulative probabilities of germination in *C. tenuiflora*

($F = 0.2316$, $P = 0.7943$). The remaining parameters (C_{50} , $TMGR$ and t_{10}) were significantly higher in the control group ($F = 5.0297$; $F = 7.0075$, $F = 12.815$; $F = 12.888$; $P < 0.05$) and no differences were identified between priming treatments (Fig. 3a). The germination response of *P. roseus* occurred during the first days of the trials and the Hill model therefore estimated high values for the α parameter. The estimated maximum cumulative germination probability was significantly higher for the control group; however, no significant differences were detected between the control and the other treatments for the remaining parameters, i.e. α , C_{50} , $TMGR$ and t_{10} (Fig. 3b).

For *C. tenuiflora*, the *DI* evaluated on the 30th day showed non-significant differences between priming treatments and the control group. However, the *DI* restricted to the 5th, 10th, and 15th days proved that priming treatments accumulated more area under the curve than the control group. The *DI* evaluated on the 5th day was higher for hydropriming than for natural priming (Fig. 4a). For *P. roseus*, the *DI* of the control group was higher than that of the hydropriming and natural priming groups, although *DI* was higher in the former than in the latter (Fig. 4b).

Field germination and natural priming in bi-monthly periods

The highest values of temperature and precipitation (Fig. 5) coincided with the highest field germination observed in both

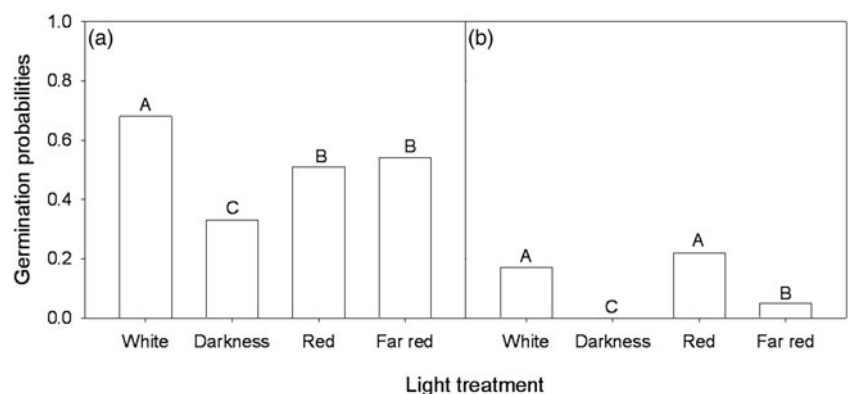


Figure 2. Germination probabilities of the seeds of (a) *C. tenuiflora* and (b) *P. roseus* under four light treatments, $n = 5$. Different letters indicate significant statistical differences within each species ($\alpha = 0.05$).

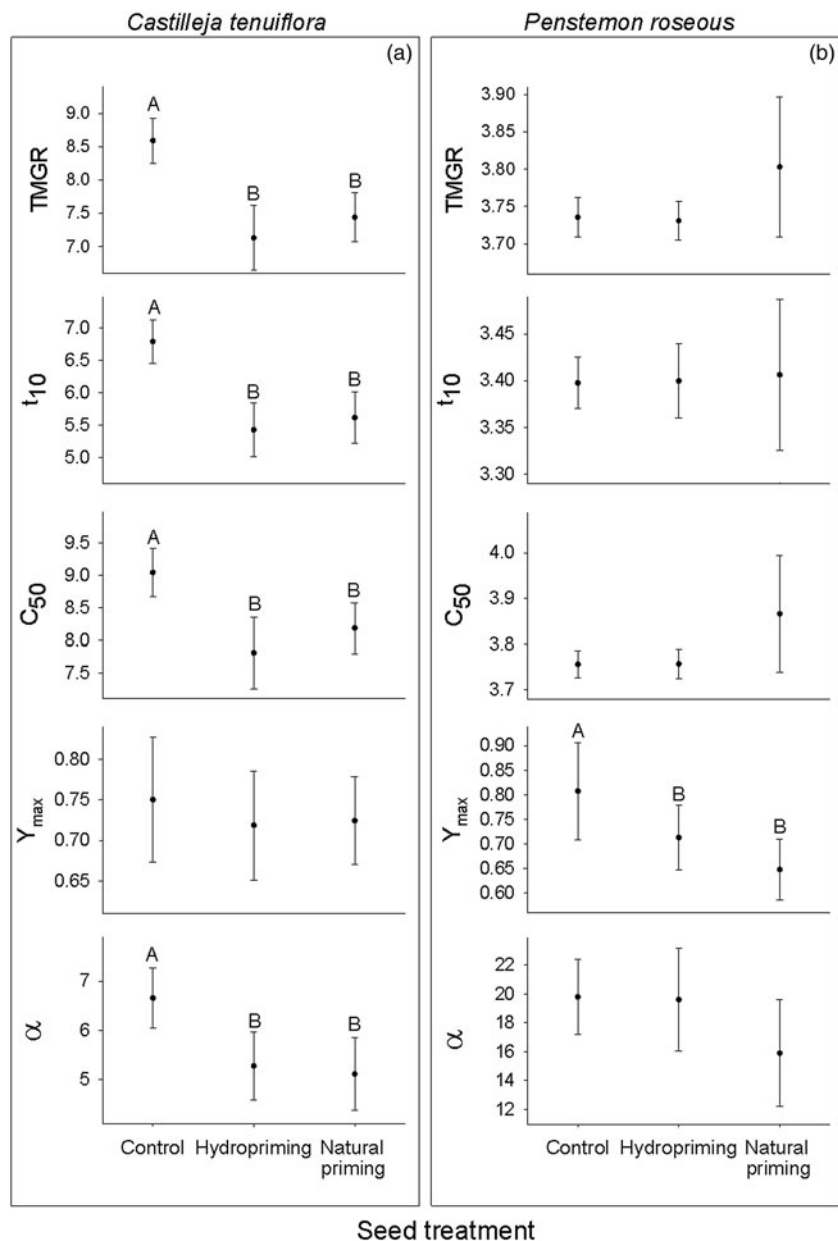


Figure 3. Germination parameters for seeds of *C. tenuiflora* (a) and *P. roseus* (b). Points show parameter means and solid black error bars correspond to 95% non-parametric bootstrap confidence intervals. Different letters indicate significant statistical differences ($\alpha = 0.05$).

C. tenuiflora and *P. roseus* (Fig. 6). After these peak values, there was a decreasing pattern in the germination probabilities of recovered seeds across the different burial periods in both species (Fig. 6). This reduction in germination probabilities was more

evident in *P. roseus* seeds after being buried for two bi-monthly periods (Fig. 6). Germination of recovered seeds decreased after the March-May period, but field germination increased in subsequent months (Fig. 6).

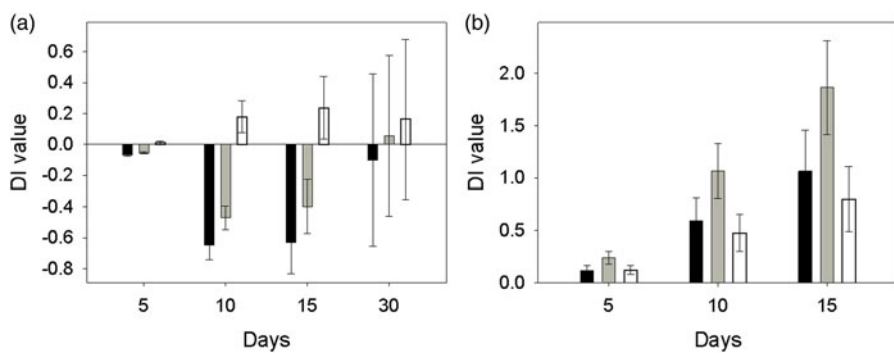


Figure 4. Dormancy index (DI) for *C. tenuiflora* (a) limited to 5, 10, 15 and 30 days and *P. roseus* limited to 5, 10 and 15 days (b), for each treatment difference (C = control, HP = hydropriming, NP = natural priming). Error bars correspond to 95% non-parametric bootstrap confidence intervals. Black bars = C-HP, grey bars = C-NP, white bars = HP-NP.

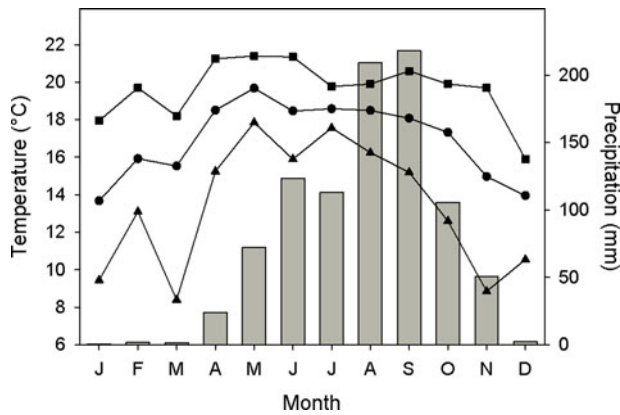


Figure 5. Climogram for 2013 for the Parque Ecológico de la Ciudad de Mexico, Mexico. Lines represent maximum (■), minimum (▲) and mean (●) temperatures; bars represent rainfall.

Castilleja tenuiflora attained the highest germination capacity in the first two bi-monthly periods ($X^2 = 28.72$, $P < 0.05$), while the other periods showed no significant differences. The α parameter was significantly higher for the September–November period than for the March–May and July–September periods ($X^2 = 15.05$, $P < 0.05$). The time required for 50% of the seeds to germinate (C_{50}) was shorter in the first three bi-monthly periods (January–July, dry season) than in the last two periods (July–November, rainy season) ($X^2 = 28.54$, $P < 0.05$). The TMGR was also shorter in the first three bi-monthly periods ($X^2 = 27.09$, $P < 0.05$). Finally, t_{10} was lower in the first two burial periods ($X^2 = 31.19$, $P < 0.05$) (Fig. 7a).

For *P. roseus*, the highest germination response was recorded for the first two periods, but no significant differences were detected in the model parameters. No germination was recorded after the third recovery period. The TMGR ($X^2 = 8.59$, $P < 0.05$) and the α parameter ($X^2 = 11.99$, $P < 0.05$) were higher for the May–July period, whereas germination capacity was significantly lower in the same period ($X^2 = 13.52$, $P < 0.05$). However, the May–July results are inferred from a very small sample and therefore no definitive conclusions can be made regarding this particular result (Fig. 7b).

Discussion and conclusions

Photoblastic response

Diversity in seed response to light has been reported in other species with small seeds that also grow in the PECM, such as

Buddleja cordata, *Chenopodium ambrosioides* and *Wigandia urens* (Vázquez-Yanes and Orozco-Segovia, 1990). Polymorphic germination response may allow the production of seedlings in different light conditions, which can be an advantage in heterogeneous habitats (Vázquez-Yanes and Orozco-Segovia, 1990) such as lava fields, where the microtopography creates diverse microenvironments (Maestre et al., 2003; Pedrero-López et al., 2016).

The germination responses of *P. roseus* and *C. tenuiflora* differed despite the fact that both species are perennial heliophytes, grow in the same environment and experience similar heterogeneous and fluctuating conditions. Priming induced fast germination (TMGR, C_{50} , t_{10} and α) in *C. tenuiflora* and did not affect germination probabilities. In contrast, priming did not promote germination in *P. roseus* and in fact reduced the germination probabilities.

A facultative root hemiparasitic habit among the Orobanchaceae (Douglas, 1973; Montes-Hernández et al., 2015), including the genus *Castilleja*, might help explain the polymorphic response of *C. tenuiflora* to light. Germination in darkness might favour the establishment of the parasitic relationship in the early development and growth of seedlings, although further research is required in order to confirm this.

Sensitivity to light in *P. roseus* suggests positive photoblastism, but there was a very low occurrence of germination to demonstrate it, probably due to physiological dormancy. Positive photoblastism prevents seed germination in the rock fissures, which are a common feature of the volcanic substrate, where seedling development is impossible due to the lack of light. Since the seeds of both studied species are small (2 mm approximately), seedlings might be unable to emerge if they are buried deeply (Wesson and Wareing, 1969). The genus *Penstemon* is normally reported as positive photoblastic, with low germination rates and complex germination patterns, but the seeds respond well when growing in growth chambers (de Mello et al., 2009).

Both species have permeable seed coats. In *P. roseus*, strong disinfection treatment was necessary due to the considerable development of fungi on the seeds during germination testing. Sodium hypochlorite solutions were used for disinfection, but these may reduce seed coat hardness (Hsiao, 1979). Disinfection probably overcame the physiological dormancy and consequently, the germination percentage in this case was higher than in the light test. Moreover, during that disinfection process, when seeds were placed in ethanol, a non-identified substance was solubilized (J. Belmont, personal observation). Liberation of germination and growth inhibitors with solvents has been reported for *Hypericum philonotis*, another species growing in the PECM,

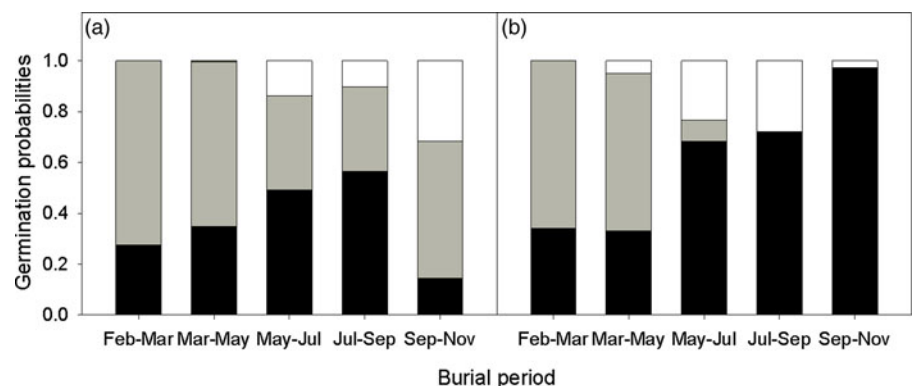


Figure 6. Germination probabilities of the seeds of *C. tenuiflora* (a) and *P. roseus* (b) in the field and of recovered seeds that experienced natural priming. Black bars = non-germinated seeds, grey bars = germination of recovered seeds, white bars = germination in the field. Non-germinated seeds indicate those that did not germinate either condition.

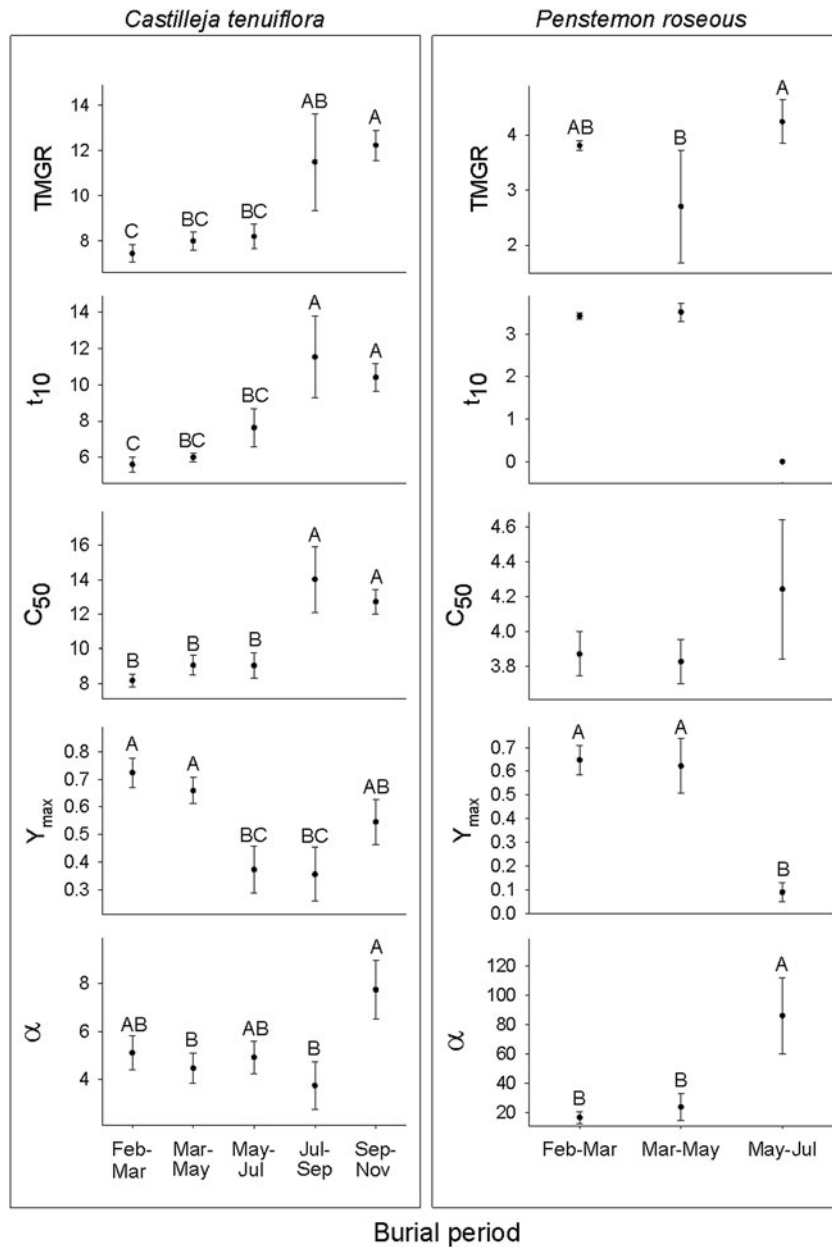


Figure 7. Germination parameters for *C. tenuiflora* (a) and *P. roseous* (b) recovered after burial in the Parque Ecológico de la Ciudad de Mexico. Different letters indicate significant statistical differences ($\alpha = 0.05$). Points show parameter means and solid black error bars correspond to 95% non-parametric bootstrap confidence intervals.

the seeds of which germinate following treatment with acetone (Sánchez-Coronado *et al.*, 2015).

While the disturbed shrublands of the PECM receive considerable annual precipitation, the basaltic substrate generates conditions of soil dryness (Cano-Santana *et al.*, 2006). A relatively hard but permeable seed coat therefore allows imbibition (but not always germination or natural priming), which could take place during the irregular spring and early summer rains that occur in the zone. Seed coat permeability of *C. tenuiflora* and *P. roseous* allows these species to exploit the random precipitation that may favour fast germination and growth when the rainy season is fully established, as has been observed in other shrubland species (Thanos *et al.*, 1995).

Priming treatments

Both priming treatments accelerated the germination of *C. tenuiflora* without affecting germination capacity. These results agree

with those reported by Badek *et al.* (2006), who found that *Callistephus chinensis* and *Solanum lycopersicum* seeds under hydropriming (24 h, unlimited water) required less time to reach 50% germination. González-Zertuche *et al.* (2002) reported the same positive effect of hydropriming in *Buddleja cordata* seeds. In contrast, priming treatments reduced germination capacity in *P. roseous*, as is the case with many horticultural species (Bray, 1995).

In comparison with the control group, both hydropriming and natural priming decreased the germination capacity of *P. roseous*. This difference could be the result of passing the 'no-return point', which occurs once the metabolic changes are activated and the seed consequently loses its capacity to endure desiccation and dies, thus producing fewer seedlings (Schopfer and Plachy, 1984). The germination curve in *P. roseous* reached its maximum during the initial days after sowing (Fig. 3b). This suggests that germination of *P. roseous* is triggered immediately as soon as suitable environmental conditions are established (Fig. 7b).

Low germination responses were obtained after the rainy season (July to September) for the seeds under natural priming. The germination capacity of *P. roseus* decreased drastically after a 6-month burial period, probably because most of the seeds had already germinated after the rainy season and the non-germinated seeds remained dormant or lost their viability. Physiological dormancy has been observed in other *Penstemon* species (Ogle and Peterson, 2013), but our results suggest that *P. roseus* seeds can remain quiescent in a transient seed bank until the soil moisture increases at the beginning of rainy season. This behaviour has been reported by González-Zertuche *et al.* (2002) in species of the PECM like *Buddleja cordata*, a species in which rapid germination of seeds at the beginning of rainy season is essential for seedling establishment due to its deep root system that taps the water trapped in the volcanic substratum. González-Zertuche *et al.* (2001) reported that occasional rains in spring and winter, along with changes in soil moisture, promote natural priming of buried seeds. The germinative patterns of *P. roseus* match the germinative behaviour of some species from the dry scrubland of the USA, such as *P. bicolor* and *P. petiolatus* (Meyer *et al.*, 1995).

In *C. tenuiflora*, the germination capacity of the seeds that underwent natural priming was lower than that of the hydropriming and control groups. However, the time required to reach the early stages of germination improved in the first burial periods (before full establishment of the rainy season), giving the seeds the opportunity to colonize this type of disturbed environment. This confers great advantages to a population that develops in a heterogeneous environment with fluctuating conditions where nutrients and water are limited (Thanos *et al.*, 1995).

Our results emphasize the versatility of *C. tenuiflora* and *P. roseus* in terms of colonizing fluctuating and heterogeneous habitats and indicate that priming plays an important role in the ability of these two native species to colonize a harsh successional environment such as the lava fields. While further information is required in order to establish the role of *C. tenuiflora* and *P. roseus* in the ongoing successional process, it is clear that both species can colonize disturbed shrubland areas, form a seed bank and efficiently respond to changing and even unpredictable conditions.

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