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Breaking seed dormancy in *Opuntia rastrera* from the Chihuahuan desert

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Abstract

In this paper, we investigated if germination of after-ripened seeds of *Opuntia rastrera* Weber could be enhanced using chemical (acid) and mechanical scarification pretreatments. *O. rastrera* seeds need an after-ripening period to germinate ($\approx 50\%$) after 1 year and no germination occurs in younger seeds. Germination of 3-year old seeds of *O. rastrera* was evaluated using sulphuric acid (H_2SO_4) for different lengths of time, and mechanical scarification treatments (dry heating at 80 °C for 12 h and washing, immersion in water for 12 h and washing, and seed coat cutting with pliers). Similar to other wild *Opuntia* species, germination of *O. rastrera* seeds was not enhanced by any scarification pretreatment, demonstrating that an after-ripening period is the best—if not the unique—way to overcome physiological dormancy for this species.

Keywords: Acid scarification; After-ripening; Mechanical scarification; Seed germination; *Opuntia*

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1. Introduction

Species of genus *Opuntia* spp. belonging to the subfamily Opuntiodeae among the Cactaceae, constitute a diverse group with both economical and ecological importance. It has been reported as a unique food source through time, extensively grown as fruit crop and forage crop in many countries (Mondragón-Jacobo and Pimienta-Barrios, 1995). Many *Opuntia* spp. are also used in medicine, live fences and in the prevention of soil erosion. Particularly, *O. rastrera* cladodes are used in the Chihuahuan Desert for human consumption and, during dry periods as cattle fodder (Nobel, 1994).

Despite their importance, there have been few studies assessing their germination behaviour because cultivation is done through cladodes planting for its simplicity (Mondragón-Jacobo and Pimienta-Barrios, 1995). However, natural populations of clonal species (e.g. all *Opuntia*) depend on sexual recruitment to maintain their genetic pools (Eriksson, 1993; Mandujano et al., 2001). Since the 1960s, it has been suggested, that some difficulties might be experienced in the germination of *Opuntia* spp. (Pilcher, 1970). Some treatments such as mechanical and acid scarification have been proved to promote the germination of the hard-seeded species of *Opuntia* (see Table 1). Different responses have been achieved with the use of several scarification pretreatments.

The need of a ripening period to promote seed germination has been detected since 1941 when Longnecker found that seeds of *O. phaeacantha* var. *discata* germinated better after some time of storage, but later studies found that germination decreased from 46% to 11% after 1 year (cited in Pilcher, 1970). On the other hand, seeds of *O. imbricata* germinated at a higher percentage when sown 1 year after their harvest (Pilcher, 1970). Potter et al. (1984) found that seeds of *O. edwardsii* and *O. lindheimeri* germinated best when stored for 1 year suggesting an after-ripening period. Also, Pendley (2001) affirms that some *Opuntia* species, specifically *O. phaeacantha* var. *phaeacantha* have primary dormancy that is removed more effectively with an after-ripening period.

Some other species, inhabiting colder deserts, may need a stratification period to obtain high germination percentages, such as *O. compressa* and *O. macrorhiza* (Baskin and Baskin, 1977), and *O. engelmannii* (Pendley, 2001), but for *O. tomentosa* (from a warmer region) seed germination was significantly reduced by stratification (Olvera-Carrillo et al., 2003).

For *O. rastrera* from Mapimi Biosphere Reserve (MBR) in Durango, an after-ripening period to promote seed germination has been demonstrated indicating that they have a primary dormancy due to embryo immaturity, as germination percentage increased with seeds of 2–3 years old (Mandujano et al., 1997). To assess if some pretreatment apart from after-ripening, could enhance germination of these seeds, acid and mechanical scarification treatments were tested to assess their effect upon germination of 3-year old *O. rastrera* seeds.

Table 1
Chemical and mechanical pretreatments used to promote germination for *Opuntia* species

Species	Scarification treatment	Germination (%)	References
O. phaeacantha var. *discata* Engelm.	Washing for 16 h	70	Pilcher (1970)
O. lindheimeri Engelm.	Soaking in water for 9 h	45	Pilcher (1970)
O. engelmannii Salm-Dyck	Soaking the seeds in a solution 0.012 M HCl for 15 min	82	Pendley (2001)
O. joconostle Weber	Mechanical scarification + imbibition in GA_3 at 40 ppm for 30 min	80	Sánchez-Venegas (1997)
O. tomentosa Salm-Dyck	Acid scarification for 90 min + GA_3 in 2 mg ml^{-1} at 24 °C	≈50	Olvera-Carrillo et al. (2003)
O. edwardsii V.E. Grant & K. A. Grant	Acid scarification with concentrated H_2SO_4 for 45 min at 30 °C	22	Potter et al. (1984)
O. discata Griffiths	Acid scarification with concentrated H_2SO_4 for 30 min at 20 °C	83	Potter et al. (1984)
O. lindheimeri Engelm.	Acid scarification with concentrated H_2SO_4 for 30 min at 30 °C	34	Potter et al. (1984)
O. phaeacantha Engelm.	Leaching + soaking seeds in 0.012 M HCl for 30 min	70	Pendley (2001)
O. auranthiaca Lind.	Soaking the seeds in a 50% sulphuric acid solution for 15 min + cut	43	Archibald (1939)
O. auranthiaca Lind.	Seed coat cut	72	Archibald (1939)

Treatments for species studied by Potter et al. (1984) show where maximum germination was obtained. All cited studies reported that germination in control seeds is lower than 20%.

2. Material and methods

Mature fruits of *O. rastrera* were collected in 1988 from at least 20 plants growing naturally at Mapimi Biosphere Reserve in Durango (26°40′N, 103°40′W, 1100 m altitude, 20.8 °C mean annual temperature, 264 mm mean annual precipitation; Montaña, 1990). Seeds were separated from the fruits, washed to remove pulp residues and dried at ambient temperature in the shade. Seeds were stored in paper bags at ambient temperature until sown 3 years later. Pilot experiments demonstrated that fresh seeds of this species do not germinate after several mechanical or chemical pretreatments (Mandujano et al., 1997; Mandujano, unpub. data).

Germination tests were performed in Petri dishes with 1% agar in distilled water and incubated in a growth chamber (CONVIRON Model I-18L, Winnipeg, Canada)

at a constant temperature of 25 °C under white light with a 12 h photoperiod. Five replicates of 20 seeds per Petri dish per treatment were used.

Seeds were subjected to nine scarification pretreatments (five to acid scarification, three to mechanical scarification and a non-treated control). Acid scarification pretreatments were done with sulphuric acid (H_2SO_4) at 98% for five lengths of time: 60, 80, 100, 120 and 140 min. Mechanical scarification pretreatments were: (a) small cut with pliers to each seed in the lateral arillus near the micropylar region, (b) dry heating at 80 °C for 12 h and soaking in five changes of tap water for 20 min each time, and (c) immersion in water for 120 min and soaking in five changes of tap water for 20 min each time.

All Petri dishes were put inside the growth chamber provided with incandescent lamps (Solar, 25 W) and fluorescent lamps (General Electric, 20 W). Germination was recorded during 90 days for all experiments when the radicle appeared.

Results obtained as percentages were arcsine-square root transformed to normalize data (Sokal and Rohlf, 1981). Differences in final germination percentage between treatments were tested for statistical significance through an ANOVA of a one-way experimental design of nine levels and five repetitions, followed by orthogonal comparisons analyses to carry out *F*-tests for comparisons between two groups. In the first analysis, one group corresponded to the seeds chemically scarified versus the control and in the second analysis, one group corresponded to the seeds mechanically scarified. All statistical analyses were done with the JMP statistical package (SAS Institute Inc., 1995).

3. Results

One-way ANOVA results showed that there are significant differences among treatments ($F_{(8,36)} = 26.93$, $p \leqslant 0.01$). No germination pretreatment gave better results than the controls (Figs. 1 and 2).

Orthogonal comparisons analysis showed that there are significant differences between scarified treatments and the control ($F_{(1,36)} = 62.42$; $p \leqslant 0.01$). Germination proportions obtained for seeds scarified for 60, 80, 100 and 120 min in concentrated sulphuric acid were significantly lower than the control where maximum germination was achieved (Fig. 1). Germination percentage with acid scarification at all concentrations was lower than 20%, while the control showed above 30%.

Orthogonal comparisons analysis showed no significant differences between the mechanically scarified seeds and the control ($F_{(1,36)} = 0.3079$; $p = 0.75$). Although there are no significant differences among treatments, maximum germination ($>40\%$) was obtained with immersion in water for 120 min followed by five washes of 20 min each in tap water (Fig. 2). The mean germination proportion for mechanical treatments was 0.37 ± 0.02 SE.

4. Discussion

Germination results obtained for *O. rastrera* in all treatments including the control are low ($\approx 50\%$), which coincides with the reports on other *Opuntia* species

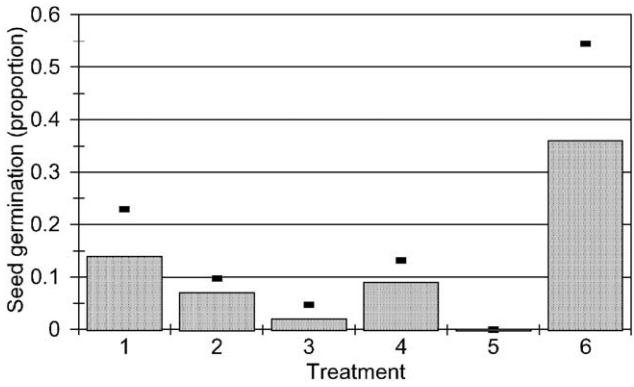

Fig. 1. Mean germination proportions for *O. rastrera* seeds (95% confidence limits, $N = 100$) at different acid germination pretreatments, where 1–5 correspond to seeds scarified for 60, 80, 100, 120 and 140 min in concentrated sulphuric acid, respectively, and 6 corresponds to the control. Mean $\pm$ SE.

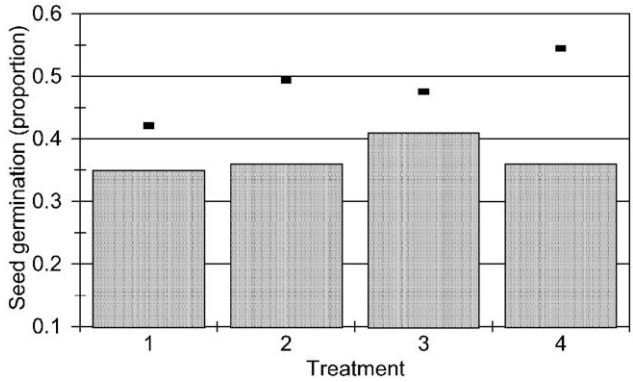

Fig. 2. Mean germination proportions for *O. rastrera* seeds (95% confidence limits, $N = 100$) at different mechanical germination pretreatments, where 1 = dry heating at 80 °C for 12 h + soaking in five changes of tap water for 20 min each time, 2 = small cut with pliers to each seed in the lateral arillus near the micropylar region, 3 = immersion in water for 120 min + soaking in five changes of tap water for 20 min each time and, 4 = control. Mean $\pm$ SE.

requiring an after-ripening period (Pilcher, 1970; Trujillo-Argueta and González-Espinosa, 1991; Nogales et al., 1999; Pendley, 2001; Gimeno and Vilà, 2002) and contrast with other species of Cactaceae that may show above a 90% germination (Rojas-Aréchiga and Vázquez-Yanes, 2000).

With respect to the germination pretreatments, our results demonstrate that acid scarification treatments applied to *O. rastrera* are harmful to the seeds because germination is reduced considerably compared to the control. Our results contrast to

other *Opuntia* species that showed improved germination response after chemical scarification pretreatments (Table 1). With respect to mechanical scarification treatments, they are not harmful to the seeds but, they did significantly enhance germination compared to the control. These results coincide with those reported by Pendley (2001) for other *Opuntia* species (Table 1), suggesting that probably some species of this genus have a physiological rather than a morphological dormancy mechanism.

According to Rojas-Aréchiga and Batis (2001), seeds of *O. rastrera* show two traits that allow them to form a seed bank: a positive photoblastism (Aguilar and Mandujano, unpub. data) and the requirement of an after-ripening period to germinate (Mandujano et al., 1997). These results support the existence of a seed bank in the study site, as it has been confirmed by Montiel and Montaña (2003) for *O. rastrera* in two habitats in the MBR. The existence and dynamics of a seed bank of *O. rastrera* has been associated with the unpredictability of the precipitation in that area, because the amount of rain determines the rate of seed production and granivore activity (Montiel and Montaña, 2003). In addition, seed dormancy has been thought to have evolved in response to unpredictable environments, leading to the existence of soil seed banks (Evans and Cabin, 1995). In *Opuntia* there are species which possess morphological (i.e., hard seed coats) (Potter et al., 1984; Olvera-Carrillo et al., 2003) and/or physiological (i.e., after-ripening) dormancy (Potter et al., 1984; Mandujano et al., 1997; Pendley, 2001). For example, the response of germination rates to storage varies between *Opuntia* species. In some cases (e.g. Pilcher, 1970; Potter et al., 1984; Pendley, 2001), germination rates are higher after storage, while in other cases (Pilcher, 1970; Olvera-Carrillo, 2003) they are lower. More studies are needed to identify evolutionary forces that are behind such different responses in this life history trait.

Similar to *O. tomentosa* (Olvera-Carrillo et al., 2003), *O. rastrera* seeds did not show an increase in germination percentage after soaking the seeds, probably because this species does not possess water-soluble inhibitors in the testa. Other *Opuntia* species reported by Pilcher (1970), such as *O. lindheimeri* and *O. phaeacantha* var. *discata*, *O. edwardsii* and *O. discata* reported by Potter et al. (1984) showed an increased germination after washing or soaking the seeds for different periods of time. For *O. rastrera*, mechanical scarification was not effective in increasing germination, which coincides with the evidence provided by Pendley (2001) for other *Opuntia* species.

Demel (1996) has suggested that the cause of different responses among different species to various presowing treatments depends on the degree of the seed coat thickness, which could explain the different results obtained under several treatments that include softening or the thinning of the testa.

It is therefore necessary to assess seed germination in wild *Opuntia* species considering that they may show either a morphological or a physiological dormancy that needs to be determined differently. In the case of a physiological dormancy, the length of the after-ripening period needs to be established, assessing seed germination of fresh seeds and after several years of seed storage under natural and controlled conditions.

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