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Seed germination of *Opuntia* species from an aridity gradient in Central Mexico

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Abstract

In the Mexican highlands there are several aridity gradients affecting *Opuntia* species composition and its biological function. Since environmental variations can affect plant traits like seed morphology as well as related processes, we conducted experiments in order to determine whether seed mass, testa hardness, seed imbibition, dormancy type, polyembryony occurrence, and different habitats are related with interspecific variation of seed germination in *Opuntia* spp. Our results showed that there were interspecific differences of seed attributes among seven *Opuntia* species along an environmental gradient in Central Mexico. Seeds of most species were able to imbibe water; however, mechanical scarification treatment did not break seed dormancy, but promoted faster germination. This pattern might be a result of the testa porosity. Seed hardness was negatively correlated with seed germination in *Opuntia jaliscana*; thus harder seeds had lower germination percentage. In contrast, seeds of *O. robusta* increased their germination percentage as seed hardness increased. We found differences in seed mass among species and localities; seeds collected in moist areas were heavier. There was evidenced a correlation of seed mass with seed germination for *O. jaliscana*. Polyembryony was present in four of the seven species studied and it appears to be more frequent in seeds from dry sites of the gradient.

Key words: *Opuntia*, testa hardness, imbibition, dormancy type, polyembryony, seed germination.

Abbreviations: *Opuntia rastrera* (Opra), *O. robusta* (Opro), *O. lasiacantha* (Opla), *O. tomentosa* (Opto), *O. jaliscana* (Opja), *O. streptacantha* (Opst) and *O. leucotricha* (Ople). Santiago, Villa de Arriaga, S.L.P. (SA), La Mesa, Ojuelos, Jal. (ME), El Molino, Lagos de Moreno, Jal. (MO), Matancillas, Ojuelos, Jal. (MA).

Introduction

Plant establishment by seed is frequently a limiting process at community level, especially in harsh environments (Flores and Jurado, 2003). Seed production and its subsequent dispersion and germination could be key factors in limiting the processes of the plant community dynamics by restricting seedling establishment in safe sites (Harper, 1977). Sexual reproduction could act as a bottleneck, if the seeds are unviable, there are unavailable dispersers or show some dormancy type (Mandujano *et al.*, 1997). These processes influence the spatial and temporal plant population dynamic and community structure (Venable and Brown, 1988).

Seed dormancy is considered a very common adaptive plant strategy in unpredictable or harsh environments, such as arid and semiarid landscapes (Jurado and Flores, 2005). Many species of the *Cactaceae* have prolonged seed dormancy (Rojas–Aréchiga and Vázquez–Yañes, 2000; Flores *et al.* 2005, 2006, 2008, 2010). Specifically, one genus of this family, *Opuntia* is considered that shows seed dormancy. The first species found to have dormant seeds was *Opuntia aurantiaca* (Archibald, 1939), although until now seed dormancy has been detected in 28 *Opuntia* species (Ochoa–Alfaro *et al.*, 2008). It has been suggested that *Opuntia* spp. seeds have both physiological dormancy (a period after–ripening to break seed dormancy) and mechanical dormancy (their embryos have a low growth potential) (Orozco–Segovia *et al.*, 2007; Delgado–Sánchez *et al.* 2010; in press), although several authors have also suggested that *Opuntia* seeds have physical dormancy (Olvera–Carrillo *et al.*, 2003; Reyes *et al.*, 2005). However, seeds with physical dormancy are water–impermeable (Baskin and Baskin, 2004), and the studies about testa hardness and imbibition in *Opuntia* species are scarce (Orozco–Segovia *et al.*, 2007).

Temporal fluctuating resources in arid environments are also limiting for the persistence of population in natural communities. Species have adaptations to respond to pulses in resources (Eriksson, 1993). Seeds with a higher germination rate could benefit early establishment of seedlings and avoid competition (Vázquez–Yañes and Orozco–Segovia, 1996; de la Barrera and Nobel, 2003).

In Mexico, *Opuntia* spp. are mainly found in the Crassicaulous matorral, which is a common vegetation type on the highlands of the Mexican states of Aguascalientes, Durango, Guanajuato, Jalisco, San Luis Potosí and Zacatecas (Rzedowski, 1957, 1966; Rzedowski and McVaugh, 1966) and they are locally called 'nopalera' (Miranda and Hernandez, 1963). In the Mexican highlands are several aridity gradients affecting *Opuntia* species composition and its biological function (Flores–Flores and Yeaton, 2000). For instance, environmental variations affect plant traits like seed morphology, as well as related processes such as seed germination (Giménez–Benavides *et al.*, 2005; Luzuriaga *et al.*, 2005; Lemos *et al.*, 2008).

Although appropriate germination response to environmental cues is partly a function of habitat characteristics and in part as a result of integration with other aspects of the species life history (Meyer and Monsen, 1991), there are not detailed studies relating habitat variation to germination differences among populations of *Opuntia* species. This lack of studies might be partially explained because asexual propagation is very common in the genus (Mondragón y Pimienta–Barrios, 1995; Reyes *et al.*, 2005). This form of reproduction is responsible for the closed–patch formation found in some nopaleras and associated communities. Therefore, it is interesting to explore if clonal propagation is affecting sexual capability in the genus *Opuntia* and its relation to environmental arid gradients. Moreover, this propagation form involves risk of diseases' transmission and loss of genetic variability (Altare *et al.*, 2006). However, both forms of reproduction are coexisting in natural populations, and sexual reproduction is needed to maintain the genetic pool (Eriksson, 1993;

Mandujano *et al.*, 2001). Furthermore, some species could be depending exclusively on sexual reproduction.

Polyembryony in *Opuntia* species has been determined (Ganong, 1898; Vélez–Gutiérrez *et al.*, 1996); however, there are not studies on polyembryony in different habitats for *Opuntia* spp., in spite of the fact that ecological conditions being essential in the induction of polyembryony (Batygina and Vinogradova, 2007).

We carried out several experiments in order to answer several questions: Are habitat variations related to germination differences among *Opuntia* species or even among populations of *Opuntia* species? What are their dormancy types? Is seed hardness related to seed germination or habitat? Is seed mass related to seed germination or habitat type? Do *Opuntia* seeds show polyembryony? If so, are differences in polyembryony bound to species and/or aridity of the habitat?

Material and methods

Study zone

We selected several plant communities along a moisture gradient (from 400 mm to up to 700 mm) in central Mexico in order to collect seeds of *Opuntia* spp. This gradient varied from dry or semiarid in the NE to temperate–subhumid in the SW sites. The dry zones include Villa de Arriaga and Mexquitic, SLP, whereas the sub–humid goes to the SW Lagos de Moreno and near Jalostotitlan, Jalisco. The historic rainfall average ranges from less than 400 mm in the NE to up to 700 mm in the SW; whereas this range was observed during the year 2008. The average temperature oscillates from 15.7 to 18.5°C; however, it was higher during 2008 (Table 1). Most of the rainfall falls during the summer months (about 60% in the NE sites and 70% in the SW zone). The hottest month is May (ranging from 28 to 30°C in the NE to 32°C at the SW sites) and the coldest is January (varying from 2 to 5°C along the gradient).

Vegetation type in the sites is mainly crassicaulescent shrub land varying in species composition (Rzedowski, 1966). It is a vegetation type with an average in height of 3 m in shrubs and trees, composed mainly of perennial woody plants, short and long–lived forbs and herbaceous species. This type of scrub named ‘nopalera’, consisting of several species of *Opuntia sensu stricto* (Miranda and Hernández, 1963; Rzedowski, 1978), covers most of the north–eastern and central parts of Mexico, and with other scrub types all together cover almost 40% of Mexico’s territory (Rzedowski, 1978). Janzen (1986) gives a good summarized description of wild “nopaleras” communities, in central Mexico. It ranges from closed monospecific stands to mosaics of mixed *Opuntia* species up to 4 m high, scattered with elements of the genus *Yucca*, *Acacia* and *Prosopis*, among other woody genera. It has been suggested that several environmental factors are involved in the control of composition and density of species, but the most significant are: relief, exposure, rock and soil type, climate (rainfall and temperature) and human management. According to Gonzalez–Espinoza and Quintana–Ascencio (1986), on moderate slope of igneous soil (Villa de Arriaga municipality) *Opuntia* (basically *O. streptacantha* and *O. robusta*) can form plant stands as dense as 1100–1200 individuals per ha. In more disturbed sites, *Opuntia* species density can range from 150–300 individuals per ha, forming more diverse stands including elements of *O. rastrera* and *O. leucotricha*.

Seeds collection

The seven species evaluated were *Opuntia rastrera* (Opra), *O. robusta* (Opro), *O. lasiacantha* (Opla), *O. tomentosa* (Opto), *O. jaliscana* (Opja), *O. streptacantha* (Opst) and *O. leucotricha* (Ople). We evaluated seed germination in all of these species, from different localities. In the particular case of *O. tomentosa* and *O. leucotricha*, it was collected seeds from only one locality because we could not find fruits in other localities throughout the gradient during the collecting field trips (Table 2).

Table 1. Average of historical records and annual average (2008) of climatic variables. Data obtained from climatic stations nearby of studied sites along the gradient (Comisión Nacional del Agua, unpublished data).

Station	Altitude (masl)	Latitude; longitude	Historical mean precipitation (mm)	2008 precipitation (mm)	Historical annual mean temperature (°C)	2008 mean temperature (°C)
Mexquitic, SLP	2062	22°16' N; 101°07' W	430.6	435.5	16.9	18.2
Villa de Arriaga, SLP	2198	21°55' N; 101°23' W	375.5	546	15.8	19.2
Ojuelos, Jal.	2175	21° 52' N; 101°37' W	590.4	716	15.7	15.3
Presa La Duquesa, Lagos de Moreno, Jal.	2165	21°44' N; 101°49' W	479.5	437.8	17.9	17.8
Lagos de Moreno, Jal.	1942	21°21' N; 101°55' W	769.1	779.1	18	17.7
Jalostotitlán, Jal.	1772	21°10' N; 102°28' W	703.2	628.5	18.5	17.9

Seeds were collected from at least 10 mature plants for each species with the purpose of include genetic variation within species. Seeds were depulped, thoroughly mixed; air dried, and storage for a 7-month period in order to after-ripen at ambient temperature before germination trials were conducted. Before germination tests, seeds were examined for: viability, hardness, imbibition capacity, and polyembryony. All germination trials were performed with normal seeds.

Seed tests

Seed viability

To examine viability, 30 seeds by species were soaked in 1% tetrazolium solution for 24 to 48 hours at 30°C. Embryos that showed a deep red color were considered viable, embryos that did not acquire a deep red color, but only light pink, were recorded as viable with low vigor (Yaklich and Kulik, 1979).

Seed germination (%)

To test the effect of seed scarification on germination, 100 seeds were scarified with a nail clipper to promote contact of the embryo with air and water. The same seed number was used for control or non-scarified. The seeds were placed in five 500 ml polystyrene containers, with 20 seeds per container, inside a greenhouse under a completely random experimental design. The substrate used was commercial greenhouse soil. No fungal inhibitors were used. The polystyrene containers were irrigated daily with 100 mm of tap water to keep the substrate moist. The temperature during test ranged from 24°C to 39.0°C; luminous intensity was between 0.31 to 37.48 lumens m⁻² and relative humidity oscilated between 30.54% to 39.21%. The number of germinated seeds was recorded thorough a 45-day period in both treatments.

Table 2. Seed collecting sites of *Opuntia* species studied. Acronyms used for sites hereafter are in parentheses.

Species / collecting site	Geographical coordinates	
	North latitude	West longitude
<i>Opuntia streptacantha</i> Lemaire /		
Santiago, Villa de Arriaga, San Luis Potosí (SA)	21°54'	101°15'
La Mesa, Ojuelos, Jalisco (ME)	21°34'	101°38'
El Molino, Lagos de Moreno, Jalisco (MO)	21°27'	101°48'
Matancillas, Ojuelos, Jalisco (MA)	21°52'	101°37'
<i>O. robusta</i> H. L. Wendland ex Pfeiffer /		
Ipiña, Ahualulco, San Luis Potosí (IP)	22°27'	101°21'
SA and MA		
<i>O. leucotricha</i> DC /		
MA		
<i>O. rastrera</i> FAC Weber /		
IP and SA		
<i>O. lasiacantha</i> Pfeiff. /		
MA		
Jalostotitlán, Jal. (JA)	21°08'	102°30'
<i>O. tomentosa</i> Salm-Dyck /		
MO		
<i>O. jaliscana</i> Bravo /		
MO, JA and		
Lagos de Moreno, Jalisco (LM)	21°18'	102°05'

Seeds were considered as germinated when the radicle emerged. Germination rate (t_{50}) was quantified counting the number of days that were required to reach 50% of the total germinated seeds (Ellis and Roberts, 1978; Sánchez *et al.*, 2005) and was calculated as follows:

$$t_{50} = \frac{\sum_{i=1}^{45} iG_i}{n}$$

Where: G_i is the number of germinated seeds observed in the i -th day and n is the total of germinated seeds in the corresponding experimental unit. The t_{50} index, also referred as mean germination time, is the number of days required to achieve a 50% of the total of germinated seeds in each experimental unit.

Seed hardness

We evaluated the seed hardness (n= 25) for each species and locality, using the Instron model 1000 (Universal Testing Instrument), serial 2791. It was applied a force of 200 kg to each seed in order to break 50% of it (Aguilar, 2003). The hardness results are reported in kilogram–force (kgf).

Seed hydration (imbibition)

After washing with tap water thirty seeds from each species and locality, they were air–dried and then weighed (initial mass) and incubated in a Petri dish (half filled with distilled water) at room temperature (22°C). Subsequently, each seed was weighed at several intervals during a 24–48 h period, until constant weight. When the weight was constant, water uptake percentage per seed was calculated as follows: $\{(final\ mass - initial\ mass) / initial\ mass\} \times 100$.

Polyembryony

In this study, polyembryony was evaluated by direct observation during the germination experiment, by counting the seeds with two or more seedlings during the seedling emergence (Mendes–Rodrigues *et al.*, 2004; Carneiro *et al.*, 2006).

Statistical analysis

A two–way ANOVA was conducted on the germination percentage of each species using locality and treatment (scarification) as factors, except for *O. leucotricha* and *O. tomentosa*, for which one–way ANOVA was conducted using treatment as factor. Another factorial ANOVA was done for germination data considering only one locality by species and treatment. All data were arc–sine transformed prior to analysis of variance (Sokal and Rohlf, 1995). In addition, we conducted one–way ANOVA with locality as factor for each species, for the variables hardness, imbibition, and t_{50} . Differences among treatments were analyzed using multiple comparisons Tukey’s test ($\alpha = 0.05$). The data that did not fulfill the ANOVA assumptions were analyzed with Kruskal–Wallis non–parametric test (Zar, 1999). All dates were analyzed using the Statistical software SAS version 9 (SAS, Inc., 1999).

Additionally, in order to consider the possible relationship between seed physical protection and seed reserve with the germination capability a Pearson correlation test for seed hardness and seed mass with germination percentage by species and locality was run.

Results

Most of the species showed high viability. The highest viability was obtained for *Opuntia jaliscana* from El Molino and *O. streptacantha* from Santiago. The lowest viability was for *O. jaliscana* from Lagos de Moreno and *O. streptacantha* from La Mesa (Table 3).

The ANOVA for germination data showed significant differences for species factor but not for treatment (F=0.88, p=0.353; Table 4). The highest germination percentage was observed in *O. jaliscana*, *O. streptacantha* and *O. lasiacantha*, and the lowest was for *O. rastrera*. The interaction effect for species x treatment was significant. In general, germination was higher in non–scarified treatment than in scarified seeds treatment, except for *O. streptacantha*. In particular, the highest germination in the un–scarified seeds was for those of *O. jaliscana*.

Table 3. Data of seed viability and vigor for *Opuntia* species studied and its collecting localities.

Species	Locality*	Viable (%)		Unviable (%)
		High vigor	Low vigor	
<i>Opuntia jaliscana</i>	MO	100	0	0
	LM	60	33.3	6.7
	JA	90	10	0
<i>O. lasiacantha</i>	MA	80	13.3	6.7
	JA	76.7	16.7	6.6
<i>O. leucotricha</i>	MA	76.7	16.7	6.6
<i>O. rastrera</i>	IP	86.7	0	13.3
	SA	96.7	3.3	0
<i>O. robusta</i>	IP	73.3	23.3	3.4
	ME	96.7	3.3	0
	MA	80	16.7	3.3
<i>O. streptacantha</i>	SA	100	0	0
	ME	56.7	43.3	0
	MO	73.3	13.3	13.4
	MA	93.3	3.3	3.4
<i>O. tomentosa</i>	MO	80	16.7	3.3

*SA, Santiago, Villa de Arriaga, SLP; ME, La Mesa, Ojuelos, Jalisco; MO, El Molino, Lagos de Moreno, Jalisco; MA, Matancillas, Ojuelos, Jalisco; IP, Ipiña, Aqualulco, San Luis Potosí; JA, Jalostotitlán, Jalisco; and LM, Lagos de Moreno, Jalisco.

Table 4. Percentage of seed germination (mean \pm standard error) in seven *Opuntia* species after two treatments (Sc: scarified and C: non-scarified seeds). The results of ANOVA statistical analysis.

Species							<i>F</i>	<i>p</i>
Opst	Opja	Opla	Ople	Opto	Opro	Opra	25.2	<0.01
56.5 \pm 5.9 ^a	52 \pm 5.1 ^{ab}	51.5 \pm 5.2 ^{ab}	35 \pm 4.9 ^{bc}	28.5 \pm 4.5 ^c	23.5 \pm 3.6 ^c	5.5 \pm 2.3 ^d		

Species x Treatment Interaction

Opja-C	Opst-Sc	Opla-C	Opst-C	Ople-C	Opla-Sc	Opja-Sc		
64.2 \pm 5.1 ^a	63 \pm 9.3 ^a	59.25 \pm 9.1 ^a	51.2 \pm 7.3 ^{ab}	44.9 \pm 5 ^{bc}	43.9 \pm 3.3 ^{bc}	40 \pm 4.5 ^{bc}		
Opto-Sc	Opto-C	Ople-Sc	Opro-C	Opro-Sc	Opra-Sc	Opra-C	3.5	<0.01
29.3 \pm 6.1 ^{cd}	27 \pm 7.1 ^{cd}	24.1 \pm 5.7 ^{cd}	23.3 \pm 5.5 ^{cd}	22.1 \pm 5.4 ^{cd}	10 \pm 3.5 ^{cd}	1.0 \pm 1.0 ^d		

Different letters indicate differences among species and species x treatments interaction ($p < 0.01$) according to Tukey HSD test. Acronyms used for species: *Opuntia lasiacantha* (Opla), *O. leucotricha* (Ople), *O. jaliscana* (Opja), *O. rastrera* (Opra), *O. robusta* (Opro), *O. streptacantha* (Opst), *O. tomentosa* (Opto).

Germination by species and locality

There was a clear effect of treatment considering the species and locality factors in this study (Table 5). In general, scarified seeds had lower germination than non-scarified seeds. The greater effect of scarification treatment was observed in *O. streptacantha* (especially in the seeds collected in SA) and the smallest in *O. rastrera*. Except for *O. rastrera*, in all the cases where locality was a factor, it had a significant effect on germination percentage. Locality was not related to differences in germination for *O. rastrera*, which had consistently low germination percentages. For *O. lasiacantha* seeds, germination was higher in the site with less rainfall (MA) than for those in a wetter climate (JA). Seed germination in *O. jaliscana* was higher in the wetter site (JA) than in the intermediate sites MO and LM.

For *O. leucotricha* and *O. robusta*, seed scarification lowered its germination considerably, almost twice than that for non-scarified seeds. In *O. rastrera* germination was positively affected by scarification treatment. In this case there were no differences between both localities. Germination was not affected by treatments in *O. tomentosa* ($F=0.14$, $p=0.7$; Table 5).

In *O. streptacantha*, we found differences among localities. Seeds collected in Santiago (SA) showed higher germination percentage, with clear differences with respect to the other three sites. The site x treatment interaction was significant for *O. robusta* and *O. streptacantha*, but with considerable overlap among the group of averages. In the dry sites (SA and MA) for *O. streptacantha*, seed scarification enhanced germination but the contrary was observed in the intermediate sites in the gradient (Table 5).

For *O. robusta* we found differences among the localities. Seeds collected at ME site had the highest germination percentage, in IP and MA sites seeds had the lowest germination. The interaction of locality x treatment was significant, even though the average difference showed considerable overlap in the formed groups. In general, for the three sites of seed origin, scarification reduced germination percentages significantly. The non-scarified seeds from ME and MA sites had the greatest germination, while the seeds from MA in Matancillas with scarification (MA-Sc) showed the lowest percentage of germination. For Ipiña seed (IP) of this species, germination was not affected by treatment (Table 5).

Seed germination of *Opuntia streptacantha*, was not affected by treatments, however, we found differences between localities, seeds of Santiago (SA), obtained higher germination percentage, than the other places. The interaction of site x treatment also showed differences, seed germination was not affected by treatments in Santiago; however, seeds germination was higher than for other zones. La Mesa non-scarified seeds had the lowest germination (Table 5).

Germination rate (t_{50})

In *O. jaliscana*, scarified-seeds germinated faster than non-scarified seeds ($F=48.6$, $p<0.01$). By localities, also we found significant differences ($F=5.52$, $p=0.01$). Seeds from Jalostotitlán germinated earlier than El Molino and Lagos de Moreno (Figure 1).

Also, in *O. robusta* scarified seeds increased germination rate ($F=10.2$, $p=0.01$). For *O. streptacantha*, non-scarified seeds were slower to germinate than scarified-seeds ($F=25.4$, $p<0.01$). Furthermore, the sites had an effect ($F=8.6$, $p=0.01$) on mean germination rate. Seeds from ME germinated more rapidly than those from MO, MA and SA (Figure 2). Mean germination rate was higher in scarified seeds than in the non-scarified for *O. tomentosa* ($F=13.0$, $p=0.03$) and *O. leucotricha* ($F=80.8$, $p<0.01$). For *O. lasiacantha* and *O. rastrera* significant differences were not detected for treatment or locality factors.

Seed hardness

Seed hardness of *O. jaliscana* was different among localities ($F=21.35$, $p<0.01$). Seeds were softer in the moist area of JA, while in LM and MO the seeds were harder. For *O. lasiacantha*, there were also differences ($F=13.8$, $p<0.01$) in seed hardness among localities. Seeds from MA were harder than those from JA (Table 6).

Table 5. Main effects and interactions on seed germination percentages of seven *Opuntia* species and their collecting sites.

<i>Opuntia jaliscana</i>						<i>F</i>	<i>p</i>		
C	Sc					5.11	0.01		
46±5.4 ^a	34±2.25 ^b								
JA	MO	LM				4.6	0.04		
52±5.12 ^a	36±5.84 ^b	33±2.9 ^b							
<i>Opuntia lasiacantha</i>									
C	Sc					14.8	<0.01		
56.0±5.13 ^a	33.0±4.43 ^b								
MA	JA					6.64	0.02		
52±5.22 ^a	37±6.0 ^b								
<i>Opuntia rastrera</i>									
Sc	C					6.99	0.02		
7±2.1 ^a	1.5±0.76 ^b								
SA	IP					0.52	>0.05		
5.5±2.3 ^a	3±1.1 ^a								
<i>Opuntia leucotricha</i>									
C	Sc					9.82	0.03		
45±5 ^a	24±5.7 ^b								
<i>Opuntia robusta</i>									
C	Sc					32.98	<0.01		
49±5.5 ^a	25±4.1 ^b								
ME	MA	IP				6.99	0.02		
53±5.2 ^a	32±3.65 ^b	24±8.3 ^b							
ME-C	MA-C	ME-Sc	IP-C	IP-Sc	MA-Sc	11.33	<0.01		
64±5.1 ^a	59±5.1 ^a	41±5.3 ^{ab}	24±5.57 ^{ab}	23±5.4 ^{ab}	12±3.4 ^b				
<i>Opuntia streptacantha</i>									
C	Sc					0.24	0.6		
39.75±3.2 ^a	38±4.7 ^a								
SA	MA	MO	ME			3.22	0.02		
56.5±5.9 ^a	37.5±4.0 ^b	34±4 ^b	27.5±4.3 ^b						
SA-Sc	SA-C	MA-Sc	MO-C	ME-C	MA-C	MO-Sc	ME-Sc	3.9	0.02
62±9.3 ^a	51±7.3 ^{ab}	44±5.8 ^{ab}	44±4.6 ^{ab}	33±6.4 ^b	31±4.3 ^b	24±3.3 ^b	22±5.1 ^b		
<i>Opuntia tomentosa</i>									
C	Sc					3.53	>0.05		
29.3±6.1 ^a	27.0±7.1 ^a								

Different letters at rows indicate differences between treatments and among locality and localities x treatment interaction according to Tukey HSD test. Acronyms used for treatments: Sc, scarified seeds; and C, control seeds. Acronyms used for localities: SA, Santiago, Villa de Arriaga, SLP; ME, La Mesa, Ojuelos, Jalisco; MO, El Molino, Lagos de Moreno, Jalisco; MA, Matancillas, Ojuelos, Jalisco; IP, Ipiña, Ahualulco, San Luis Potosí; JA, Jalostotitlán, Jalisco; and LM, Lagos de Moreno, Jalisco.

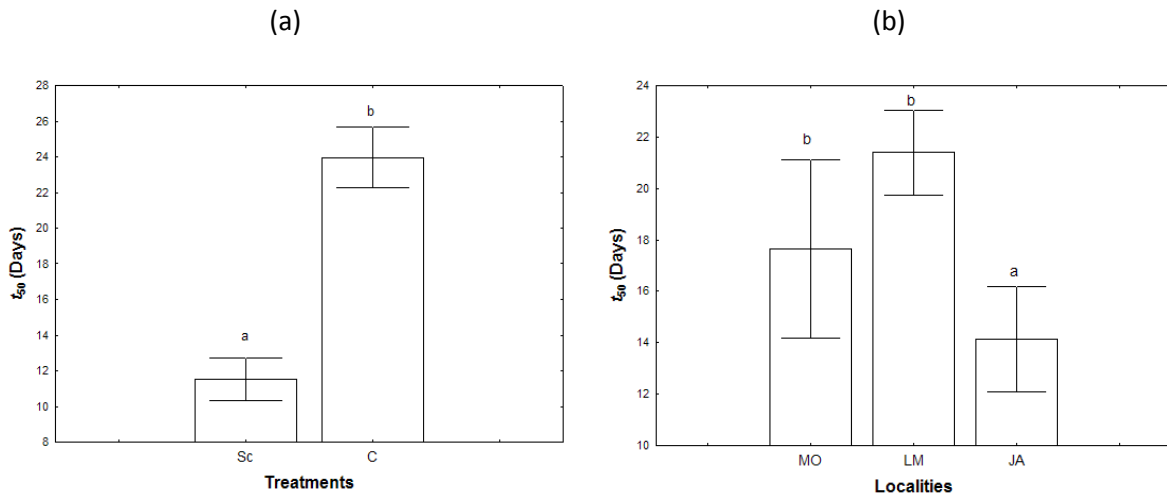


Figure 1. Mean (\pm SE) germination rate (t_{50}) for *O. jaliscana*: (a) seed treatments effect (Sc, scarified seeds; and C, control seeds); and (b) collecting sites (El Molino, MO; Lagos de Moreno, LM, and Jalostotitlán, JA). Different letters indicate significant differences ($p < 0.05$) between averages according to Tukey HSD test.

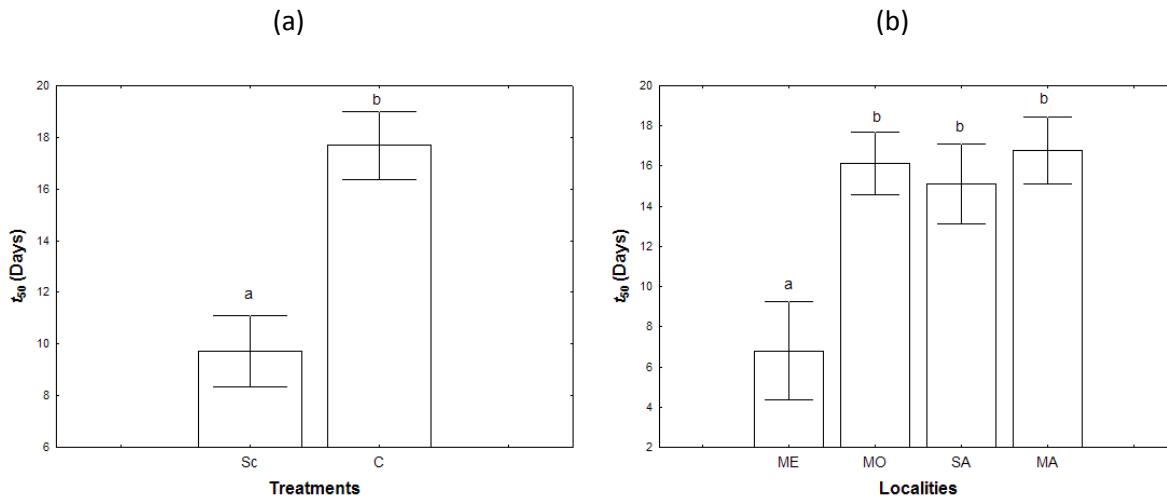


Figure 2. Mean (\pm SE) germination rate (t_{50}) for *O. streptacantha* as related to: (a) seed treatments (Sc: scarified seeds, C: control seeds), and (b) collecting sites (La Mesa, ME; El Molino, MO; Santiago, SA, and Matancillas, MA). Different letters indicate significant differences ($p < 0.05$) among averages according to Tukey HSD test.

Seeds of *O. streptacantha* were also different ($F=11.74$, $p<0.01$) in hardness by locality. Seeds from ME were harder than those from MA. Seed hardness in *O. robusta* were different by site ($F=30.7$, $p<0.01$). Seeds of MA were harder than those from IP and ME. For *O. rastrera*, seeds from SA were harder ($F=9.23$, $p<0.01$) than those from IP (Table 6). For species *O. tomentosa* and *O. leucotricha*, we did not conduct an ANOVA because only one locality was included for each species (Table 6).

For *Opuntia robusta*, correlation between seeds hardness and germination was high ($r=0.65$, $p<0.01$), indicating that harder seeds tend to show greater germination percentage (Figure 3), also correlation between seeds hardness and seeds imbibitions was significant ($r=0.3$, $p=0.01$), indicating that the harder seeds tend to imbibe more water. For *O. jaliscana*, a reverse relation was found in the form of negative correlation ($r=-0.65$, $p=0.01$) between hardness and percentage of germinated seeds. This is indicative that seed germination percentage is reduced as hardness increases (Figure 3).

Seed imbibition

Significant differences were found in seed imbibition by localities in the following species (Table 6). In *O. jaliscana*, the seeds from JA imbibed less water than seeds from LM and MO ($F=13.50$, $p<0.01$). Seeds of *O. robusta* from MA imbibed more water than seeds from ME and IP ($F=8.66$, $p<0.01$). In *O. streptacantha*, seeds from the moist zone at ME and MO imbibed less water than those in drier localities MA and SA ($F=13.43$, $p<0.01$). The other species (*O. lasiacantha* and *O. rastrera*) did not show differences (Table 6).

Seed mass

For *O. jaliscana*, it was found differences in seed weight between localities ($p<0.01$, Kruskal–Wallis test). Seeds from MO were heavier than those from JA. Also, there was a negative correlation between seed mass and seed germination ($r= -0.72$, $p<0.01$).

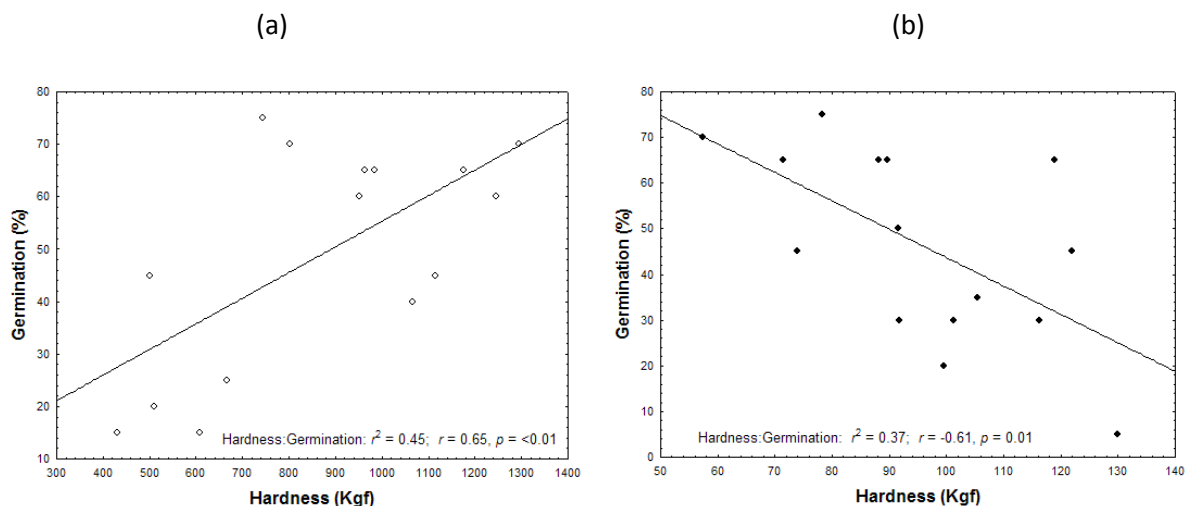


Figure 3. Correlation between seed hardness and seed germination for (a) *O. robusta* and (b) *O. jaliscana*

For *O. robusta*, the seeds from MA were heavier than those from IP ($p < 0.01$). In *O. lasiacantha*, the seeds from JA were heavier than seeds from MA ($p < 0.01$). Seeds of *O. streptacantha* from ME had more biomass ($p < 0.01$) than those from MA. Seeds of *O. rastrera* from all localities had similar seed mass ($p > 0.05$; Table 6).

Table 6. Seed hardness, imbibition rate and biomass (mean \pm SE) of seven *Opuntia* species from different localities.

Species	Locality*	Hardness (kgf)	Imbibition rate (mg)	Weight (mg)
<i>O. lasiacantha</i>	MA	76.17 \pm 3 ^a	1.7 \pm 0.05 ^a	8.9 \pm 0.27 ^b
	JA	61.5 \pm 2.1 ^b	1.6 \pm 0.03 ^a	10.5 \pm 0.24 ^a
<i>O. rastrera</i>	IP	96.9 \pm 6.7 ^b	2.66 \pm 0.14 ^a	20 \pm 3 ^a
	SA	119.1 \pm 4.6 ^a	2.75 \pm 0.16 ^a	16 \pm 4 ^a
<i>O. robusta</i>	IP	64.7 \pm 4.6 ^c	3.16 \pm 0.12 ^b	13 \pm 4 ^b
	MA	109.2 \pm 5.9 ^a	3.94 \pm 0.13 ^a	18 \pm 0.6 ^a
	ME	94.7 \pm 2.8 ^b	3.23 \pm 0.16 ^b	13 \pm 0.5 ^b
<i>O. streptacantha</i>	MA	78.6 \pm 3.05 ^c	2.6 \pm 0.05 ^{ab}	10 \pm 0.26 ^c
	ME	103.5 \pm 2.9 ^a	2.14 \pm 0.09 ^c	15 \pm 0.5 ^a
	MO	94.7 \pm 2.4 ^{ab}	2.3 \pm 0.06 ^{bc}	11 \pm 0.27 ^c
	SA	85.7 \pm 3.4 ^{bc}	2.65 \pm 0.06 ^a	12.5 \pm 0.21 ^b
<i>O. jaliscana</i>	JA	78.9 \pm 2.3 ^c	2.2 \pm 0.1 ^b	13 \pm 2.6 ^b
	LM	93.8 \pm 3.1 ^b	2.7 \pm 0.06 ^a	16 \pm 0.4 ^a
	MO	104.58 \pm 2.8 ^a	3.0 \pm 0.08 ^a	16 \pm 0.3 ^a
<i>O. tomentosa</i>	MO	103.66 \pm 3.27	2.46 \pm 0.12	16.56 \pm 0.29
<i>O. leucotricha</i>	MA	57.3 \pm 2.8	1.8 \pm 0.05	9.5 \pm 0.27

Different letters within species indicate locality differences, according to the Tukey HSD test.

*SA, Santiago, Villa de Arriaga, SLP; ME, La Mesa, Ojuelos, Jalisco; MO, El Molino, Lagos de Moreno, Jalisco; MA, Matancillas, Ojuelos, Jalisco; IP, Ipiña, Aqualulco, San Luis Potosí; JA, Jalostotitlán, Jalisco; and LM, Lagos de Moreno, Jalisco.

Polyembryony

We found low percentages of polyembryony in four of the seven species studied with no differences ($p > 0.05$) among them. *O. lasiacantha* from MA had 20.2 \pm 7.33% polyembryonic seeds and the same species from JA had 2.9 \pm 2.9%; *O. robusta* from MA had 20.7 \pm 7.6%, from ME had 8.5 \pm 4.3% and from IP had 7.0 \pm 4.7%; *O. jaliscana* from LM had 16.9 \pm 6.9% and from JA had 10.5 \pm 5.43%, and *O. streptacantha* from SA had 11.2 \pm 3.5% and from MA had 6.14 \pm 1.93%.

Discussion

Higher seed germination is usually associated to higher seed viability (TeKrony *et al.*, 1980). However, although most of the studied species had medium–high seed viability (56–100%) they also showed seed dormancy. We found interesting results, which are very helpful to answer several questions about *Opuntia* seed germination:

Are habitat variations related to germination differences among Opuntia species or even among populations of Opuntia species?

Seed dormancy is a very common adaptive plant strategy in unpredictable and harsh environments, such as those of arid and semiarid zones (Jurado and Moles, 2003; Jurado and Flores, 2005). We hypothesized that *Opuntia* seed germination is lower and slower in drier than in humidity zones. This was confirmed for *Opuntia robusta* and *O. jaliscana*, where seeds from moisture localities had higher germination than those from a drier locality. However, we found contrary results for *O. lasiacantha* and *O. streptacantha*. Thus, there is no general pattern for the studied species. Our results indicate that there are differences in *Opuntia* species in seed germination, as well as differences in germination rate, seed imbibition, seed mass, seed hardness, and polyembryony.

What are the Opuntia dormancy types?

All the studied species showed seed dormancy (seed germination <70%; Flores and Jurado, 2006). We reject the hypothesis that *Opuntia* seeds have physical dormancy (Reyes *et al.*, 2005, Olvera-Carrillo *et al.*, 2003), since seeds from all studied species showed imbibition. However, although all seeds were imbibed, scarification treatment did not break seed dormancy (although promoted faster germination), and non-scarified seeds germinated more than the scarified seeds.

Is seed hardness related to seed germination or habitat?

It has been suggested that seed coat (testa) functions as regulator in imbibition (Souza and Marcos-Filho, 2001), and that a rapid and high percentage of germination is associated with a thin testa (Maiti *et al.*, 1994). In fact, high testa hardness has been associated with low germination in *Opuntia* species (Stuppy, 2002). These results agree for *O. jaliscana*, but not for *O. robusta* where harder seeds had higher seed germination, which might be explained based on the porosity of the seed coat, because we also found a positive correlation between seed imbibition and seed hardness. We speculate that this behaviour is explained considering that well developed embryos might break hard testas. In these habitats hard seed testas are needed for their establishment in harsh environments and aggressive dispersers (Janzen, 1986). There was no correlation between testa hardness and seed germination for *O. streptacantha*, *O. lasiacantha* and *O. rastrera*. Seed hardness was not affected by the habitat (locality), contrary to findings for other species (Meyer and Monsen, 1991).

Are seed mass related to seed germination or habitat type?

It is considered that variation in seed mass is a critical trait in seed dispersal and seedling establishment (Hendrix *et al.*, 1991), and that it is determined by both genetic of the species and environmental conditions (Winn and Werner, 1987). We found differences in seed mass among and inside species. We found a negative correlation between seed mass and seed germination, but just in *O. jaliscana*. Seeds of *O. jaliscana* were heavier in the dryer site than in the moist site, but in *O. robusta*, *O. lasiacantha* and *O. streptacantha* an opposite pattern was observed. These findings may indicate that *Opuntia* seeds from harsh environments are lighter.

Do Opuntia seeds show polyembryony? If so, are differences in polyembryony bound to species and/or aridity habitat?

We found polyembryony in four *Opuntia* species (*O. jaliscana*, *O. lasiacantha*, *O. streptacantha*, and *O. robusta*). Polyembryony appears to be more frequent in *Opuntia* seeds from dry sites. It could also be related with minimal temperature variation, particularly in the locality of Matancillas, which have the lowest minimum temperatures (2°C) in the studied gradient, and here we found higher polyembryony percentage. This is in agreement with Batygina and Vinogradova (2007), who suggested that, high or low temperatures as well as changes in environmental conditions and low and high humidity could induce polyembryony.

Conclusions

All the studied *Opuntia* species had seed dormancy, nevertheless the seeds of all the species were imbibed, and therefore scarification treatment did not break seed dormancy. The scarification promoted faster seed germination. These results indicate that seeds from wetter localities had higher germination capability than those from a drier locality, and especially this was observed for *O. robusta* and *O. jaliscana*. However, opposite results were observed for *O. lasiacantha* and *O. streptacantha*. Testa hardness was negatively correlated with seed germination for *O. jaliscana* and positively for *O. robusta*. It was found differences in seed mass within species. The seeds were heavier in wetter than in drier areas. It was identified negative correlation between seed mass and seed germination in *O. jaliscana*, but not in other species. In *Opuntia jaliscana*, *O. lasiacantha*, *O. streptacantha*, and *O. robusta*, polyembryony was found and this suggested that it is most frequent in drier than in wetter sites.

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