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Germination Responses of *Opuntia* spp. to Temperature, Scarification, and Other Seed Treatments[1]

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Abstract. Scarification in sulfuric acid consistently increased germination of *Opuntia edwardsii* sp. nov, *O. discata* Griffiths, and *O. lindheimeri* Engelm. #[3] OPULI seeds over that of untreated seeds. Optimum constant temperatures for germination were generally 25 to 35 C and germination was not enhanced by alternating temperatures. There was a trend for increased germination following leaching in water for 12 h which suggested the presence of chemical germination inhibitors. Seeds passed through the digestive tracts of cattle exhibited average germination percentages that were 1.5 times greater than seeds removed from ripe fruits.

Additional index words. Opuntia edwardsii, Opuntia discata, Opuntia lindheimeri, pricklypear, OPULI.

INTRODUCTION

Fleshy fruited *Opuntia* spp. form a widespread complex of at least 16 species in the southern United States and northern Mexico (1). *Opuntia* provides valuable cover and forage for wildlife and has been used as emergency feed for livestock in Texas (2). Fruits and cladophylls of *Opuntia* are consumed by deer, cattle, and goats (5). Lundgren et al. (2) estimated that 28% of rangeland in Texas had light to dense stands of *Opuntia* spp. However, 40% of these stands occur in the Edwards Plateau region of Texas where *Opuntia* is generally considered by ranchers to be detrimental to livestock health. Most livestock health problems resulting from animals eating *Opuntia* occur in sheep and goats, but health problems in cattle have also been reported (3).

There is a paucity of information on the autecology of *Opuntia*. Germination studies yield basic ecological information on requirements for propagation by seeds and subsequent development and establishment of seedlings. Such information can enhance development of more effective control practices by determining when germination occurs so biological, chemical, or mechanical control practices may be properly timed. Also, knowledge of temperatures and seed treatments necessary for germination assesses the germination potential of seeds. This information provides insight of the potential for establishment or reinvasion into uninfested areas or areas on which pricklypear has been controlled. This study was initiated to determine, in controlled environments, the germination requirements of three common species of *Opuntia* in western Texas.

MATERIALS AND METHODS

Three seed lots were collected in the fall of 1979 and five in the fall of 1980 for use in germination trials. The seeds of two of the 1979 collections were separated from the fruits after the fruits were allowed to air dry at room temperature (20 C) for several weeks (AD = air dried). Seeds of the other 1979 lot were removed from the fruits after they had overwintered outdoors on the soil surface (OW = overwintered). The seeds of all but one of the 1980 lots were removed from ripe fruits, washed, and oven dried (OD = oven dried) at 55 C. The seeds of the other 1980 lot were removed from fresh cattle feces and oven dried at 55 C (IG = ingested). Soil surface temperatures frequently reach 55 C during summer in western Texas, thus we feel that there was no biologically significant effect due to oven drying the seeds. Seeds from *O. edwardsii* (1979 AD and 1980 OD) and *O. discata* (1980 OD) were collected in Tom Green County, Texas. Seeds from *O. lindheimeri* were collected in Tom Green County (1979 AD, 1979 OW, and 1980 OD) and Coleman County (1980 OD and 1980 IG). Seeds were stored indoors in paper sacks at room temperature (20 C) until the germination trials started.

Initial germination trials were conducted from January to mid-February 1981 and involved six seed treatments plus an untreated control which were evaluated under six constant temperature regimes for 14 days. Seed treatments were 72 h imbibition at room temperature (20 C), boiling in distilled water for 1 h; or scarification in concentrated sulfuric acid for 15, 30, 45, or 60 min. Constant temperature regimes were 10, 15, 20, 25, 30, and 35 C. Subsamples of seeds from each of the eight lots were subjected to all seed treatments except the *O. lindheimeri* IG lot. Subsamples of seeds subjected to acid treatments were weighed before and after treatment to determine percent weight loss.

Based on results from the initial study, additional trials were conducted from July through December 1981 utilizing only the five 1980 lots. Nine seed treatments plus an untreated control were evaluated under five constant and four alternating (16 h/8 h) temperature regimes for 28 days. Seed treatments were 48 and 96 h imbibition, 12 and 24 h leaching, placing seeds in boiling water and allowing them to cool to room temperature over a period of 45 to 60 min, or 30-, 60-, 90-, or 120-min soaking in concentrated sulfuric acid under constant agitation. Imbibition treatments were conducted at room temperature (20 C) by placing about 20 g of seeds in 50 ml of distilled water for the appropriate length of time. Leaching treatments were conducted by placing about 20 g of seeds under running tap water (50 l/h at 25 to 30 C) for the appropriate length of time. Temperature regimes were 15, 20, 25, 30, and 35 C constant, and 25/10, 25/15, 30/10, and 30/15 C alternating regimes.

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[3] WSSA-approved computer code from Important Weeds of the World, 3rd ed., 1983. Available from WSSA, 309 West Clark St., Champaign, IL 61820.

Five replications of 25 seeds for each seed treatment were placed in controlled environmental chambers for each temperature trial in both studies. Seeds for each replicate were placed on filter paper in a petri dish to which 8 ml of distilled water were added. Petri dishes were placed on moist paper towels in closed glass boxes. The imbibition and leaching treatments were terminated just prior to the start of a trial. All seeds were germinated in the dark but were periodically exposed to light when germination was evaluated. All seed were treated for 1 min with 0.5% (w/v) sodium hypochlorite solution to control fungi and were then rinsed thoroughly with tap water before being placed in the petri dishes. Germination was checked daily during the 14-day trials and weekly during the 28-day trials. Additional water was added to the dishes as needed. Seeds in both studies were considered to have germinated when the length of the extruded radicle or cotyledons exceeded the length of the seed (2.6 to 4.4 mm).

Analyses of variance were conducted on percent germination transformed by $\sin^{-1}$. Analyses were conducted separately for each accession in each study by a split-plot design, with temperature regime as the whole-plot effect and seed treatments as the subplot effect. Five replications (dishes) for each seed treatment within temperature regimes formed the nested interactions for the error terms. If no germination occurred for a given seed treatment at all temperature regimes tested, this treatment was excluded from the analysis. Similarly, if no germination occurred under a given temperature regime for all seed treatments tested, this temperature regime was excluded from the analysis. Mean separations were conducted by LSD tests at the 5% level. Treatments and temperatures which produced low germination are excluded from the tables for brevity. Taxonomic nomenclature of *Opuntia* follows the recent revisions by Grant and Grant (1).

RESULTS

O. edwardsii. Germination of *O. edwardsii* was generally low during the 14-day trials (Table 1). For the 14-day trials, germination of seeds that were collected in 1980 was 2% or less in all treatments and temperatures (data not shown). Maximum germination of seeds collected in 1979 (22%) occurred at 30 C following 45-min acid scarification. Germination of the 1979 seeds was significantly greater at 30 C than at other temperatures. Either 45- or 60-min acid scarification produced significantly greater germination than any other seed treatment tested for the 1979 seeds of *O. edwardsii*.

For the 28-day trials, constant temperature regimes of 30 or 35 C produced the greatest germination response for the 1980 *O. edwardsii* seeds (Table 1). Alternating temperature regimes did not significantly enhance germination over that of optimum constant temperatures (data not shown). Maximum germination (10%) occurred at 35 C following 30-min acid scarification. The 30- or 60-min acid scarification treatments significantly increased germination of *O. edwardsii* seeds over that of untreated seeds or those which received any other seed treatment.

Table 1. Germination of two accessions of *O. edwardsii* seeds collected in Tom Green County, Texas. Seeds collected in 1979 were removed from air dried fruits and tested in 14-day trials. Seeds collected in 1980 were removed from ripe fruits and tested in 28-day trials.

Seed treatment[a]	Temperature regime (C)[a] 20	25	30	35	Avg.
			(%)		
			1979 seeds		
Control	0	0	1	1	<1
15-min acid	1	1	3	1	1
30-min acid	2	1	5	2	2
45-min acid	1	6	22	5	9
60-min acid	1	13	18	15	12 LSD_r=4
Avg.	1	4	10	5	$\bar{x}$ = 7
				LSD_c=5	LSD_i=9
			1980 seeds		
Control	0	0	1	0	<1
30-min acid	3	2	6	10	4
60-min acid	0	2	1	6	1 LSD_r=1
Avg.	<1	1	2	2	$\bar{x}$ = 1
				LSD_c=1	LSD_i=3

[a]Exclusion of data for any seed treatments or temperature regimes means very low or no germination occurred. However, averages are over all means used in the statistical analysis. LSD_r, LSD_c, and LSD_i apply to the row averages, column averages, or the interaction means, respectively (P = 0.05).

O. discata. More seeds of *O. discata* germinated over a wider range of temperatures and seed treatments than those of any other accession (Table 2). Maximum germination during the 14-day trials (48%) occurred at 25 C following 60-min acid scarification. Optimum constant temperature regimes were 25 and 30 C during the 14-day trials whereas 30 and 35 C were optimum regimes during the 28-day trials (Table 2). Acid scarification of 30 min or longer significantly increased germination above that of untreated seeds during the 14-day trials. Maximum germination during the 28-day trials (83%) occurred at 20 C in seeds that were acid scarified for 30 min, but this was not significantly different than that which occurred at 15, 25, 30, 25/15, or 30/15 C temperature regimes following 30-min acid scarification. In addition to the 30- and 60-min acid scarification treatments, the leaching treatments generally increased germination of *O. discata* seeds over that of untreated seeds at 25, 30, and 35 C constant temperature regimes. However, 12- or 24-h leaching treatments did not significantly increase germination over that of untreated seeds when averaged over all temperature regimes (Table 2).

O. lindheimeri. Maximum germination of the 1979 AD seeds of *O. lindheimeri* during the 14-day trials occurred at 25 C (21%) following 60-min acid scarification but this was not significantly different from that which occurred at 30 C (18%) (Table 3). The optimum constant temperature regime for the 1979 AD seeds was 30 C during the 14-day trials.

Table 2. Germination of *O. discata* seeds during 14-day and 28-day trials. Seeds were removed from ripe fruits collected in the fall of 1980 in Tom Green County, Texas.

Seed treatment[a]	Temperature regime (C)[a] 15	20	25	30	35	25/10	25/15	30/10	30/15	Avg.
	(%)									
14-day trials										
Control	0	0	2	4	1	...	...	...	...	1
30-min acid	0	14	21	31	0	...	...	...	...	11
45-min acid	1	22	35	29	6	...	...	...	...	16
60-min acid	0	42	48	39	15	...	...	...	...	25 LSD_r=4
Avg.	<1	16	22	23	5	...	...	...	...	$\bar{x}$=8
									LSD_c=4	LSD_i=9
28-day trials										
Control	0	3	14	36	35	0	13	3	16	14
48-h imbibition	0	4	4	20	12	0	2	2	4	5
12-h leaching	0	6	27	62	50	3	12	0	8	19
24-h leaching	0	4	31	43	30	9	9	2	6	15
30-min acid	74	83	77	74	59	68	79	51	78	72
60-min acid	32	64	55	30	45	46	55	25	50	45
90-min acid	9	6	10	11	42	14	15	6	7	14
120-min acid	6	13	12	6	2	6	18	3	10	9 LSD_r=5
Avg.	14	23	27	33	32	17	24	11	22	$\bar{x}$=21
									LSD_c=4	LSD_i=13

[a]Exclusion of data for any seed treatments or temperature regimes means very low or no germination occurred. However, averages are over all means used in the statistical analysis. LSD_r, LSD_c, and LSD_i apply to the row averages, column averages, and interaction means, respectively (P = 0.05).

Table 3. Germination of two accessions of *O. lindheimeri* seeds collected in Tom Green County, Texas. Seeds collected in 1979 were removed from air dried fruits and tested in 14-day trials. Seeds collected in 1980 were removed from ripe fruits and tested in 28-day trials.

Seed treatment[a]	Temperature regime (C)[a] 20	25	30	35	Avg.
	(%)				
1979 seeds					
15-min acid	2	0	3	2	1
30-min acid	0	2	4	2	2
45-min acid	2	6	8	7	5
60-min acid	9	21	18	6	11 LSD_r=3
Avg.	3	7	8	5	$\bar{x}$=5
				LSD_c=3	LSD_i=6
1980 seeds					
Control	0	0	2	0	<1
30-min acid	14	27	34	13	12
60-min acid	8	6	2	1	3 LSD_r=2
Avg.	3	5	6	3	$\bar{x}$=2
				LSD_c=2	LSD_i=5

[a]Exclusion of data for any seed treatments or temperature regimes means very low or no germination occurred. However, averages are over all means used in the statistical analysis. LSD_r, LSD_c, and LSD_i apply to the row averages, column averages, and interaction means, respectively (P = 0.05).

Acid scarification for 60 min significantly increased germination of the 1979 AD seeds above that of any other seed treatment. Germination of the 1979 OW seed was 2% or less for all seed treatments and temperature regimes tested during the 14-day trials (data not shown). The two seed lots collected in 1980 exhibited very similar germination during the 14-day trials (data not shown). Maximum germination within both lots (10%) occurred at 25 C following 60-min acid scarification (data not shown). No seeds from the 1980 IG lot germinated during the 14-day trials (data not shown).

All seed lots of *O. lindheimeri* collected in 1980 had similar germination responses to temperature during the 28-day trials (Tables 3 and 4). However, the responses of these seed lots differed with respect to seed treatments. The optimum constant regimes for the OD lots were 30 and 35 C for the Tom Green and Coleman County lots, respectively (Tables 3 and 4). Alternating temperature regimes did not significantly increase germination of the OD lots above that of the optimum constant temperature regimes. Germination of the Coleman County IG lot was greatest at 35 C but this was not significantly different than that which occurred under the 25/15 C alternating temperature regime (Table 4). Acid scarification for 30 min significantly increased germination of the 1980 Tom Green County accession over all other seed treatments (Table 3), whereas germination of the Coleman County OD lot was significantly increased by 30-, 60-, or 90-min acid scarification (Table 4).

Acid scarification for 60 min resulted in the highest germination of the Coleman County IG lot (Table 4). The 12-h leaching treatment tended to increase germination of both OD lots over untreated seeds, but this increase was only significant for the Coleman County OD seeds at 25 and 30 C (Table 4). Leaching did not affect germination of the seeds of *O. lindheimeri* that had passed through the digestive tract of cattle (data not shown).

DISCUSSION

The acid scarification treatments in the 28-day trials were more severe than those of comparable duration in the 14-day trials because seeds for the 28-day trials were constantly agitated whereas seeds for the 14-day trials were only occasionally stirred in the acid. However, the consistency with which acid scarification increased germination indicates that an impermeable seed coat prevents rapid germination of pricklypear seeds.

Acid scarification may increase germination by reducing physical and/or chemical dormancy mechanisms. Pricklypear seedcoats are extremely hard, suggesting a physical barrier to imbibition of water, which may be overcome by chemical scarification. Percent dry matter loss of the seeds averaged over the seven seed lots tested in the 14-day trials was 24, 32, 38, and 43 percent for the 15-, 30-, 45-, and 60-min acid treatments, respectively. This suggests that reduction of the seedcoat mass (thickness) may account for increased germinability.

Reduction of the seedcoat mass by acid treatment may also destroy chemical germination inhibitors. Pilcher (4) reported 70% germination of *O. discata* seeds following 16-h leaching in water. We also observed a trend for increased germination of *O. discata* and *O. lindheimeri* following the leaching treatments. These results suggest the presence of chemical germination inhibitors in *Opuntia*.

The differences in the way the seeds were handled in 1979 compared to 1980 restricts the validity of any comparisons between years. However, seeds of *O. edwardsii* (Table 1) and *O. lindheimeri* (Table 3) collected in Tom Green County in 1979 generally had higher germination percentages than those collected in 1980. The summer of 1980 was unusually hot and dry, which may have reduced the viability of the year's seed crop. However, reduced germination of the 1980 accessions compared to that of the 1979 accessions also suggests the possibility of an afterripening requirement for these species. The high germination percentages for *O. discata* during the 14-day trials, which were conducted only 2 months after the seeds were collected, suggests the absence of any afterripening requirement for this species.

An intraspecific comparison of drying methods is only possible with the two 1979 lots of *O. lindheimeri*. Seeds from fruits which had overwintered on the soil surface (data not shown) had lower germination percentages than seeds removed from ripe fruit the previous fall and air dried (AD) (Table 3). This suggests that germinability may be reduced when seeds dry within intact fruits. However, if fruits are ingested by animals they are gleaned of most of the pulpy fruit mass when deposited in feces.

Table 4. Germination during 28-day trials of two accessions of *O. lindheimeri* seeds collected in 1980 in Coleman County, Texas. Uningested seeds were removed from ripe fruits. Ingested seeds were recovered from cattle feces.

Seed treatment[a]	Temperature regime (C)[a]								Avg.
	15	20	25	30	35	25/10	25/15	30/15	
	(%)								
Uningested seeds									
Control	0	0	0	4	1	2	0	0	1
12-h leaching	0	0	10	14	1	1	0	0	3
30-min acid	0	6	21	18	24	0	1	3	8
60-min acid	4	12	9	14	27	0	3	4	8
90-min acid	2	15	10	5	33	7	14	4	10 LSD_r=2
Avg.	1	4	7	7	10	2	3	2	$\bar{x}$ = 4
								LSD_c=2	LSD_i=6
Ingested seeds									
Control	0	0	0	5	0	0	0	0	1
30-min acid	2	3	13	25	33	1	6	12	11
60-min acid	10	30	28	37	34	12	48	31	26
90-min acid	1	11	10	6	29	10	18	6	11
120-min acid	2	2	0	2	0	2	13	2	3 LSD_r=3
Avg.	2	5	7	9	12	3	10	6	$\bar{x}$ = 6
								LSD_c=2	LSD_i=9

[a]Exclusion of data for any seed treatments or temperature regimes means very low or no germination occurred. However, averages are over all means used in the statistical analysis. LSD_r, LSD_c, and LSD_i apply to the row averages, column averages, or the interaction means, respectively (P = 0.05).

Timmons (6) reported that total germination of *O. macrorhiza* seeds ingested by jackrabbits (*Lepus californicus*) increased by 50% compared to seeds removed from ripe fruits. In this study, germination of *O. lindheimeri* seeds recovered from cattle feces (6%) was 1.5 times greater than seeds removed from ripe fruits (4%). Maximum germination of the seeds collected from cattle feces (48%) was almost 1.5 times greater than maximum germination of uningested seeds (33%).

Results of these laboratory studies suggest *Opuntia* seeds may germinate when soil temperature exceed 25 C. In western Texas, surface soil temperatures are generally above 25 C from April to October. However, there are occasional days during the November to March period when surface soil temperatures reach this level. *Opuntia* seeds are generally slow to germinate. Usually 4 to 7 days are required for any germination to occur, and maximum germination usually does not occur for 2 weeks or longer. This suggests that ample soil water may be necessary for periods of a week or longer before germination will occur. Although no tests of the duration of viability of *Opuntia* seeds were made in this study, the impermeable nature and apparent presence of chemical germination inhibitors in the seed coats of these species suggests that pricklypear seeds may remain dormant in the soil for considerable periods of time. Therefore, pricklypear may reinfest areas from seed where mature stands have been controlled. Also, as is the case with many species of undesirable plants, wild and domestic animals may disseminate *Opuntia* seeds to uninfested pastures, or pastures on which it has been controlled. These seeds, thus disseminated, have greater germinability than seeds not passed through animal digestive systems.

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