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## BREAKING SEED DORMANCY IN THE WHITE SAXAUL TREE (*HALOXYLON PERSICUM* BOISS. ET BUHSE) AMARANTHACEAE

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□ Seed dormancy is an obstacle to revegetation and reclamation efforts, particularly in arid and semiarid environments. Therefore, the objective of this study was to determine the most effective germination pretreatment for *Haloxylon persicum*, a tall desert shrub or small tree. The experiment employed a completely randomized block design. Dormancy breaking treatments included scarification with 98% sulfuric acid for 10, 20, 30, and 60 minutes; debracting seeds; debracting + piercing seeds; stratification for 1, 2, 3 and 4 weeks; and leaching seeds in flowing water for 1, 2, 3, and 4 days. Results demonstrated that scarification with 98% sulfuric acid for 10 min was the most effective treatment which increased germination from 23.3% (control) to >82.6%.

**Keywords:** *Haloxylon persicum*, revegetation, seed dormancy breaking, sulfuric acid

### INTRODUCTION

Two broad approaches to revegetation in arid and semiarid environments are seeding and transplanting. Seeding is a common revegetation practice in natural resources management because it has a low initial expense. Unfortunately, seeding is less reliable when climatic conditions, particularly rainfall, are variable (Anderson and Ostler, 2002).

Revegetation of deserts increases water quantity and quality, thus triggering or enhancing socioeconomic opportunities at the national and international level. Native species are often required because they are either adapted to the conditions that will exist at the site after revegetation, or

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because they are disturbance adapted and perform well during the first few years (Bainbridge et al., 1998).

A key species in desert revegetation efforts is *Haloxylon persicum* Bunge ex Boss. et Buhse. Like many members of the Amaranthaceae, *H. persicum* is a stem-succulent xerophytic shrub that grows only in non-saline sandy desert soils (Song et al., 2006). *Haloxylon persicum* has traditionally been used as livestock feed and for firewood; therefore it is crucial to protect this species in desert lands (Tobe, et al., 2000). As with other species used for revegetation, establishment of *H. persicum* is constrained by poor germination and seedling emergence. Therefore, an understanding of the requirements to break dormancy and initiate germination of a potential revegetation species is necessary to maximize production from the often limited native seed supply in many restoration efforts (Middleton, 1999).

Influences of pretreatment on seed germination enhancement and breaking seed dormancy for desert and other species have been reported and studied by many authors (Commander et al., 2009; Fedrico and Mollard, 2009; Nosrati et al., 2008; Soleiman et al., 2009; Soyler and Khawar, 2006; Sun et al., 2009; Tang et al., 2009; Zare et al., 2011).

Preliminary studies demonstrated high seed dormancy for *H. persicum*. Therefore, the objective of the present study is to determine the most effective treatment at breaking dormancy. It is our goal to find a method that is both cost and time efficient as an initial step in desert revegetation and reclamation. We hypothesize that pretreatments can break seed dormancy and increase *H. persicum* germination compared to untreated seeds.

## MATERIALS AND METHODS

Seeds of *Haloxylon persicum* were collected from 15–16 September 2009 from the salty soil on the shallow sandy dunes at Naevin, Isfahan Province (33° 44'N, 50°, 48'E). Long term (1975–2003) annual average maximum and minimum temperatures are 23.4°C and 9°C, respectively. Long term mean precipitation has been 121.1 mm per year (IR of Iran Meteorological Organization, 2010). Preliminary studies demonstrated that *H. persicum* flowers in the early spring, and seeds reach maturity by the autumn to be dispersed from November to December. However, the seeds are dormant upon dispersal, so this experiment studied different dormancy pretreatments. To prevent fungal infection, seeds were surface sterilized with 5% sodium hypochlorite for two minutes, and then rinsed in distilled water before pretreatments.

### Scarification Pretreatments and Germination Conditions

Pretreatments included scarification with 98% sulfuric acid for 10, 20, 30, and 60 minutes. Seeds were also debracted and placed under optimal germination conditions in a dark germinator at 25°C. A similar but more

mechanically abrasive pretreatment was debracting the seeds as well as piercing the seed coats using a scalpel. Another pretreatment included imbibing seeds in water for 4 hours, and then stratifying them for 1, 2, 3 and 4 weeks at 6°C in a dry Petri dishes before placing them under optimal germination conditions. As a final group of pretreatments, seeds were leached in flowing water for 1, 2, 3, and 4 days. There were a total of 15 different treatment groups. The control group consisted of non-treated seeds. For each group there were three replicates of 25 seeds.

Seeds were placed in Petri dishes on Whatman No. 1 filter paper within 10 cm plastic Petri dishes with a tight fitting lid and containing 10 mL distilled water. Petri dishes were placed in plastic bags to reduce moisture loss, and then randomly placed in a dark germinator (model 2848; Cleland International, Inc., Rogers, MN, USA) at 25°C.

### Germination Determination

Germination was recorded every other day for 20 days. Seeds were counted as germinated when the emerging radicle was at least 2 mm in length (AOSA, 1991). Characteristics of final germination, cumulative germination, and first and final germination day were calculated. In addition, germination rate was estimated using a modified Timson's index of germination velocity ( $\sum G/t$ ) where  $G$  equals the percentage of seed germination at 2 day intervals, and  $t$  is the total germination period (Khan and Ungar, 1984). The maximum value possible using this index with our data was 62.5 (i.e., 1250/20).

### Statistical Analysis

Statistical analyses were based on randomized block design. Germination data were arcsine transformed before statistical analysis to ensure homogeneity of variance. Data were subjected to a one-way ANOVA using the MSTAT-C program (MSTATC, Michigan State University, East Lansing, MI, USA). Treatment means were compared via the Duncan's multivariate test at a 5% level.

## RESULTS

One-way ANOVA results showed that all treatments had a significant effect on final percent germination and germination rate ( $P \leq 0.01$ ; Table 1).

Final percent germination and germination rate results showed that all treatments significantly increased percent germination and the germination rate of *H. persicum* (Table 2) compared to the control. All treatment groups except 1 week of stratification, 1 day of leaching, and the control decreased the number of days it took for the seeds to first germinate (Table 2). None of

**TABLE 1** Effects of seed dormancy breaking treatments on germination characteristics of *H. persicum*

Source	Degrees of freedom	Final germination day	First germination day	Germination rate index	Final germination day
Replication	2	7.27	0.47	0.20	39.82
Treatments	14	6.01*	8.62*	26.47*	756.36*
Error	28	1.79	0.37	5.67	80.97
CV	—	8.96	18.28	15.54	15.54

\*are significantly different at 1%.

the treatments significantly affected the number of days till final germination (Table 2).

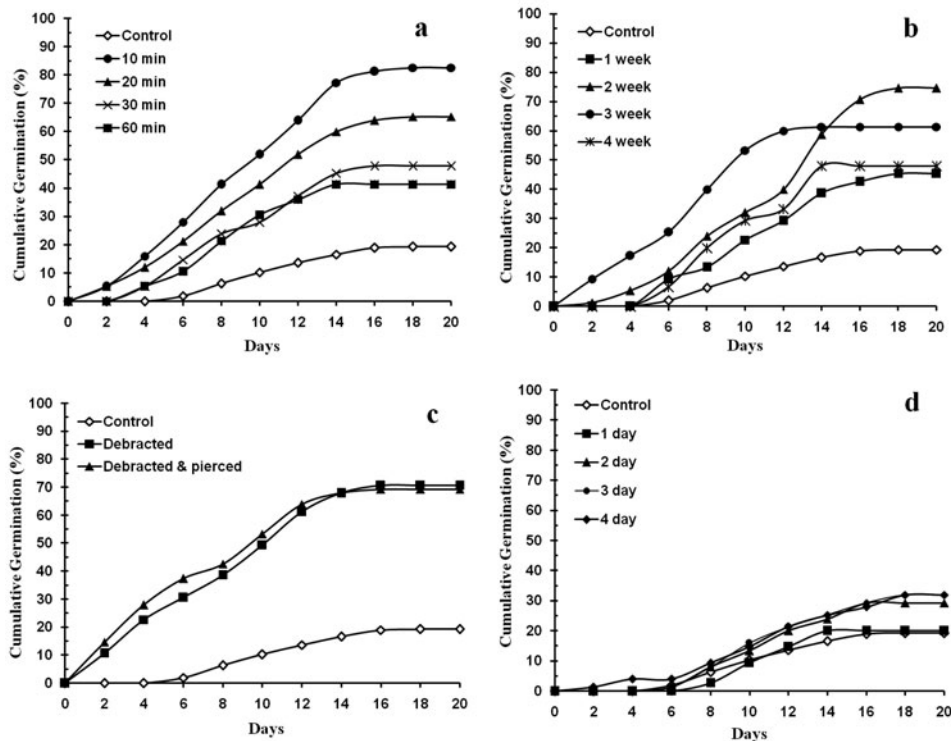
### Effect of Sulfuric Acid Treatments

Sulfuric acid significantly increased percent seed germination and germination rate of *H. persicum*. All four sulfuric acid time treatments (10, 20, 30, and 60 minutes) increased final germination percent and germination rate compared to the control. However, the 10 minute treatment had the greatest increase in cumulative percent germination from 23.33% (control) to 82.67% (Figure 1a), and germination rate index from 14.5 to 51.6 (Table 2). Sulfuric acid treatments decreased the number of days until seeds

**TABLE 2** The effects of different seed dormancy breaking treatments on germination characteristics in *H. persicum*

		Germination characteristics			
Treatment		Final% germination	Germination rate index	First germination day	Final germination day
H <sub>2</sub> SO <sub>4</sub> (minute)	10	82.7 a	51.7 a	2.0 d	14.0 bcd
	20	65.3 bc	40.8 bc	2.0 d	15.3 abc
	30	57.3 cde	35.8 cde	3.6 bc	16.0 ab
	60	41.3 e	25.8 e	2.0 d	13.3 cd
Stratification (week)	1	45.3 de	28.3 de	6.0 a	15.3 abc
	2	74.7 ab	46.7 ab	2.6 cd	17.3 a
	3	61.3 bcd	38.3 bcd	2.0 d	12.6 d
	4	48.0 de	30.0 de	4.0 b	15.0 abcd
Leaching (day)	1	45.3 de	28.3 de	6.0 a	15.3 abc
	2	74.7 ab	46.7 ab	3.3 bc	17.3 a
	3	61.3 bcd	38.3 bcd	2.0 d	12.6 d
	4	48.0 de	30.0 de	4.0 b	15.0 abcd
Debracted		70.7 abc	44.2 abc	2.0 d	15.3 abc
Debracted & pierced		69.3 abc	43.3 abc	1.6 d	15.3 abc
Control		23.3 f	14.6 f	6.6 a	14.0 bcd

Different letters indicate that germination characteristics are significantly different at 1% level based on Duncan's multivariate test.



**FIGURE 1** Cumulative percent germination vs. number of days for *H. persicum* seeds A) after being exposed to 98% sulfuric acid treatments for up to 60 minutes, B) after being exposed to stratification treatments for up to 4 weeks, C) that have been debracted or debracted and pierced with a scalpel, and D) that have been leached for up to 4 days in flowing water.

germinated (Table 2), but had no effect on the final day of germination (Table 2).

### Effect of Stratification Treatments

Seed stratification significantly increased final percent germination and germination rate (Figure 1b). Even though all stratification treatments increased final percent germination and germination rate, seeds that were stratified for moderate amounts of time (2 and 3 weeks) demonstrated a greater increase in germination compared to the shortest (1 week) and longest (4 weeks) stratification treatments. With the exception of week one, stratification decreased the number of days until seeds germinated (Table 2).

Only 3 weeks of stratification had a significant effect on the final day of germination (Table 2).

### Effect of Debracting Treatments

Final percent germination (Table 2) and germination rate (Table 2) increased significantly due to debracting. Seeds that were debracted and had their seed coats pierced also had a higher final percent germination and an increased germination rate compared to the control (Table 2). These treatments decreased the number of days until germination, but the final day of germination was not affected (Table 2). There were no significant differences between debracted or debracted and pierced treatment groups for any of the parameters measured (Figure 1c).

### Effect of Leaching Treatments

All leaching treatments resulted in a significant increase in final percent germination (Table 2) and germination rate (Table 2) compared to the control. However, 1 day of leaching had no effect on decreasing the number of days until seeds first germinated (Table 2). Two and 4 days of leaching caused an intermediate decrease in germination time, and 3 days of leaching had the greatest decrease in time to first seed germination (Table 2). Only the 2 day treatment significantly decreased the final day of germination compared to the control (Table 2), and as an overall trend 2 days of leaching had the greatest effect on seed germination (Table 2 and Figure 1d).

## DISCUSSION

Acid scarification is known to be highly effective at improving germination of other species with hard seed coats (Shaltout and EL-Shorbagy, 1989), and the results of this experiment corroborated these findings.

The 98% sulfuric acid treatment was fully effective in breaking dormancy of *H. persicum* at 10 min. Similar results were reported by Grouzis and Danthu (2001), Kukarni et al. (2006) and Zare et al. (2011). For species like *H. persicum* with hard seed coats, the resistance of the seed integument to the penetration of water may be alleviated by a sulfuric acid treatment, manual or mechanical scarification, or by scalding with hot water. These treatments reduce the resistance and impermeability of the integument (Elberse and Breman, 1989).

Stratification for 2 weeks, leaching for 2 days, debracting, and debracting + piercing the seed coats increased the Timson's Index and first day of germination, although not to the same degree as the acid treatment. Our finding concurs with Uzen and Aydin (2004) and Soyler and Khawar

(2006) who found that the most effective treatment in breaking the hard seed dormancy of legume seeds was mechanical disruption.

This experiment demonstrated that mechanical scarification broke seed dormancy. It is therefore believed that *H. persicum* is exogenously dormant. Seeds with exogenous dormancy are not permeable to water and gas because of a hard seed coat, so imbibition cannot occur which decreases or prevents germination. When imbibition cannot occur, turgor pressure and radicle growth is not enough to rupture the seed coat and therefore break seed dormancy (Bewley, 1997). Exogenous dormancy can also result from inhibitory chemicals in the seed coat (Black and Bewley, 2000).

The 98% sulfuric acid treatment for 10 min. demonstrated to be a very practical way to break dormancy in *H. persicum*. The hypothesis that seed dormancy of *H. persicum* can be broken through different pretreatment methods was supported. Moreover, we feel our goal in finding a cost and time efficient method in breaking seed dormancy in *H. persicum* has been met because treating seeds with sulfuric acid is simple and cost effective for land managers.

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