Improvement of Seed Germination in *Atriplex repanda* Phil.

SERGIO LAILHACAR-KIND AND HORTON M. LAUDE

Highlight: *Sereno saltbush (*Atriplex repanda *Phil.) is a valuable browse producer in arid coastal regions of central Chile. Direct seeding has been impractical using the heavily indurated fruits, which in laboratory germination tests yield zero to 2%. Among treatments which have been reported, manually clipping off the bracts has been the most beneficial. Debracted fruits which had not germinated would do so once the testa was ruptured. Bract removal without rupturing the testa was ineffective. Virtually 100% germination was obtained after fruits had been debracted and the testa pierced without damage to the embryo which encircles the endosperm. Germination approaching 10% was obtained from 3000-utricle samples after treatment in a modified small legume-seed scarifier which broke the pericarp and freed the seed. Higher values appear possible and the technique may have application to other small fruits with hard coverings.*

Sereno saltbush (*Atriplex repanda* Phil) is an evergreen shrub endemic to arid regions of Chile, primarily from 30° to 32° south latitude. Annual precipitation in this Mediterranean-type climate varies from 100 to 300 mm during the rainy period of 4 to 6 months. The species appears to be favored by the higher humidity of coastal areas and possesses the ability to absorb water through its leaves (Arentsen, 1972). The plant grows to a meter in height and branches profusely with flexible and leafy stems. During the critical period from late spring to early fall when annual vegetation is dry, Sereno saltbush maintains a satisfactory protein level and produces green forage much desired for browse.

The potential for greater use of this plant in Chile and elsewhere in arid to semiarid climates is presently limited by very low seed germination. Whereas a number of *Atriplex* species may be expected to give from 15 to 80% germination in laboratory tests without seed treatment, only 0 to 2% has been reached in repeated trials with Sereno saltbush. Until germination is improved, little progress can be made on studies of seeding and establishment. To date the information on establishment and utilization has been obtained mostly from seedlings transplanted to the field from the nursery or greenhouse.

The objectives of this investigation were to determine more precisely the causes for the poor seed germination in Sereno saltbush and to attempt to identify a type of treatment adaptable to the preparation of seed in quantity for direct seeding.

Sereno saltbush produces a one-seeded indehiscent fruit called a utricle. The principal parts of this fruit are shown in Figure 1. Two winged bracts enclose the seed in a locule or cavity. These bracts are fused at the base and together form the pericarp. They are extremely indurated and are not abraded, cut, or broken easily. The testa, embryo, and endosperm comprise the true seed, and it is this that is termed the seed in this paper. Once the bracts are removed, the seed is released, but it is difficult to remove the hard bracts without injuring the embryo which curves around the endosperm like a ring. Therefore it is the entire fruit which is harvested and usually sown.

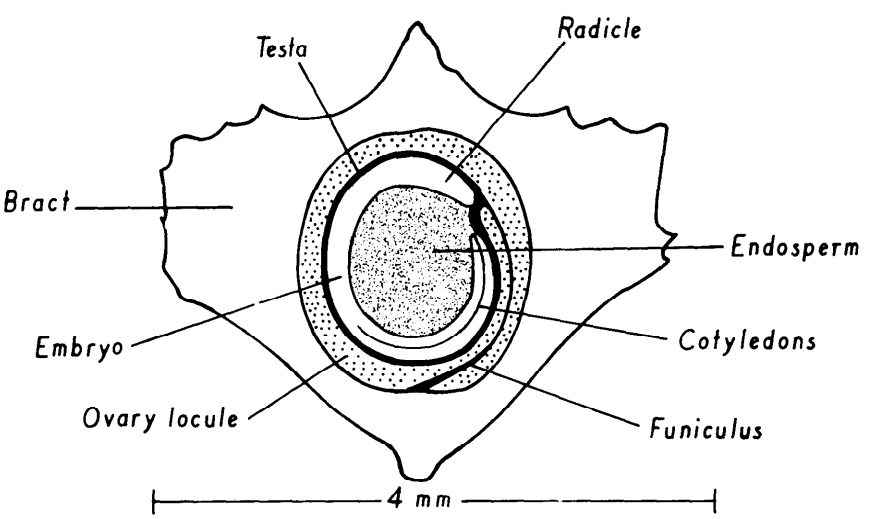

Fig. 1. *Diagrammatic sketch of the utricle in a lateral longitudinal section.*

Authors are graduate student and professor of agronomy, Department of Agronomy and Range Science, University of California, Davis.

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Two recent Chilean publications on Sereno saltbush have provided information useful to this study. Cristi and Gastó (1971) reported the effect on germination of a number of treatments applied to the utricles. An 18-day germination test revealed little to no benefit from immersion in running tap water or in hot water, from fire, or scarification. Forty-five minutes in sulfuric acid proved to be the best chemical treatment, yielding a germination of 28.6%. Debracting the fruits by removing the entire pericarp using fingernail clippers was superior and gave germinations above 70%. Leighton (1972) established that Sereno saltbush was among the lowest in germination of 6 species of *Atriplex* that he compared. Three were native to Chile (*A. atacamensis, A. coquimbana,* and *A. repanda*), two to Australia (*A. leptocarpa,* and *A. nummularia*), and one to North America (*A. canescens*). He also compared several treatments to improve germination in Sereno saltbush and obtained the highest germination, 68%, with debracted fruits. These studies have indicated a considerable impairment to germination associated with the bracts. Our studies have implicated other structures as well.

Materials and Methods

The Sereno saltbush fruits used in this investigation were kindly provided by the University of Chile and the Corporation for Development of Production (CORFO), having been collected in the provinces of Santiago and Coquimbo in north-central Chile. The utricles were treated 6 to 8 months after collection. Only the larger fruits appearing fully developed and heavier than 6.5 mg were used as controls and in the following treatments:

Debracted—bracts cut with a razor blade and removed from the seed.

Partially debracted—one wing trimmed with a razor blade until the ovary locule was reached, but the seed including the testa remained uncut and in the locule.

Testa pierced—small tangential cut through the testa with razor blade without damage to embryo after total or partial bract removed.

Testa intact—no break observable in testa under 40-fold magnification.

Sulfuric acid—lots of 50 utricles were immersed in 20 ml of H_2SO_4 sp gr 1.834 for 45 to 60 minutes or sp gr 1.625 for 60 to 80 minutes, then washed in running tap water for 30 minutes and blotted dry.

Sodium hypochlorite—lots of 50 utricles were immersed for 9 to 15 hours in 20 ml of fresh household Clorox (5.25% NaClO), washed in running tap water for 30 minutes and blotted dry.

The germination chamber operated in darkness at 20°C for 10 hours alternating with 12°C for 14 hours. These simulate the mean temperatures during the germination season in the area of adaptation. Twenty-five seeds were equally spaced on germination blotters 20 by 25 cm in size, were then covered with a second moist blotter, and both blotters were rested on an inclined plexiglass plate. The bottom edge of the blotters contacted distilled water in a reservoir and served as a wick to supply moisture for germination. This technique is an adaptation of the slanted-substrate method described by Jones and Cobb (1963). The covering blotter was periodically sprayed with the fungicide Captan to control contamination during the germination period which extended to 56 days in some cases. In initial trials a seed was considered germinated and was removed from the blotters when the combined length of radicle and cotyledons exceeded 10 mm. Later this required length was reduced to 5 mm, since it was observed that most seedlings of this size were capable of continued growth. Upon removal from the blotters, the seedlings were placed on a steam-sterilized mixture of moist soil and sand, and nearly all became established to be used later for transplanting.

Except for the percentages in Table 3, treatment means were based on 50-seed lots (the total of two slants) replicated in 4 or 6 blocks. The data were transformed to arcsine values and analyzed by Duncan's Multiple Range test for statistical comparison.

Results and Discussion

Germination of Sereno saltbush has been improved by sulfuric acid treatment of the utricles and by mechanical removal of the pericarp (Cristi and Gastó, 1971). In view of their results, we tried two immersion durations in each of two concentrations of sulfuric acid and manually debracted utricles with a razor blade. In addition we included several intervals of immersion in sodium hypochlorite, which is easier to handle than sulfuric acid and yet is effective with some plants (Laude, 1951), and also a treatment of debracted fruits which showed visible damage to the testa. The latter was included since we had previously observed an instance in which 30% of the debracted seeds which had not germinated in 33 days, did so within 12 additional days once the testa was intentionally ruptured. The results are presented in Table 1. Neither chemical treatment appeared to be feasible when compared to debracted seeds with the testa pierced. Three questions at least are raised by these results. Is the germination of debracted seeds attributable to a cutting of the seedcoat incidental to the debracting procedure? Must the entire pericarp be removed or will partial debracting suffice? Can debracting be

Table 1. Germination (%) following manual or chemical treatment to weaken or remove the pericarp of mature utricles.

Treatment of utricle		% germinated[1] after 24 days
Control (entire utricle)		0.0
Debracted		32.0
Debracted and testa pierced		93.0
Sulfuric acid (immersed)		
(Sp gr 1.834)	45 min.	0.0
	60 min.	0.0
(Sp gr 1.625)	60 min.	0.0
	80 min.	1.0
Sodium hypochlorite[2] (immersed)	9 hr.	0.0
(5.25% NaClO)	11 hr.	1.0
	13 hr.	4.0
	15 hr.	17.0

[1] Mean of four 50-seed replications.
[2] Household Clorox.

Table 2. Influence of debracting and of piercing the testa on germination (%).[1]

Days in germinator	Control	Testa intact[2]		Testa pierced	
		Partially debracted	Debracted	Partially debracted	Debracted
7	0.0 h[3]	0.0 h	0.0 h	0.0 h	3.7 h
14	0.0 h	0.3 h	0.3 h	9.7 g	79.3 d
21	0.0 h	0.3 h	0.7 h	24.3 f	95.7 b
24	0.0 h	0.7 h	1.0 h	32.3 f	96.3 ab
28	0.0 h	0.7 h	1.0 h	49.3 e	97.7 ab
35	0.0 h	1.0 h	1.0 h	70.3 d	98.3 ab
42	0.0 h	1.0 h	1.0 h	80.0 cd	99.0 ab
49	0.0 h	1.0 h	1.3 h	87.0 c	99.3 a
56	0.0 h	1.0 h	1.7 h	91.3 bc	99.3 a

[1] Mean of six 50-seed replications.
[2] Testa integrity verified by examination under 40-fold magnification.
[3] Factorial analysis of time and treatment effects by Duncan's Multiple Range test. Any two means not having a letter in common differ significantly at 0.01 level.

accomplished mechanically in such a way that the testa is pierced and yet a sufficient number of embryos remain undamaged?

The results presented in Table 2 pertain to the first two of these questions. Even though exacting care was exercised during the debracting process, it was necessary to ascertain whether or not the testa had been pierced. This was determined by first scrutinizing the debracted seeds under 10-fold magnification and then by examining those still of uncertain condition under 40-fold magnification. Accordingly, the "testa intact" and "testa pierced" columns of Table 2 are presented with considerable assurance of accuracy. Whether or not the fruit is partially or fully debracted, it is evident that germination is negligible and not significantly different from the control if the testa remains intact. With the testa cut through, either degree of debracting yielded over 90% germination and in the case of the fully-debracted, reached 99.3%. We would suggest that the germination reported for debracted utricles in earlier studies may reflect the amount of inadvertent testa rupturing or piercing. Indeed, before we appreciated the importance of testa condition, we obtained germination in our debracted seedlots ranging from 24 to 70%. A comparison of the partially and fully-debracted treatments, both with testa pierced, suggest a delay in germination when part of the pericarp remains with the seed. After partially debracting the fruit, the seed still is encased by bract tissue. This delay, therefore, may be attributable to mechanical impairment by the remaining bract or to some germination-inhibiting substance in the bract, or to both. We did not investigate this further.

How to debract the utricles in quantity and to pierce the testa, yet to keep embryo damage to a minimum, is still largely unresolved. Springfield (1970) has used hammermilling to dewing fruits of *A. canescens*. He reported that total germination was not increased but faster germination and greater ease in planting resulted. Our quantity of fruits was inadequate to try this method. Graves et al. (1974) used a legume-seed scarifier designed for small seedlots on *A. canescens* and *A. polycarpa*. This scarifier consisted of a brass cylinder 5.4 cm in diameter by 7.5 cm in length, lined with emery cloth over which the seeds were driven by compressed air. Although they varied the air pressure, time, and coarseness of emery cloth, this method did not benefit germination in *A. canescens* and did produce some damage to embryos in *A. polycarpa*.

We tried this same scarifier but with modification on the more indurated utricles of Sereno saltbush. The emery cloth was withdrawn, exposing the brass surface of the cylinder, and air at 40 psi was used to strike the utricles against that metal surface. This succeeded in breaking the pericarp and in freeing the seed from the locule of some fruits. We termed these seeds "peeled" to distinguish them from those debracted with a razor blade. Starting with lots of 3000 utricles in the scarifier cylinder we found that an average of 12.5% were peeled after 100 seconds of continuous operation and 23.4% were peeled after 400 seconds. Seeds peeled during this processing continued to churn within the cylinder and were subject to abrasion of the testa and breakage of the embryo. After processing, the seeds were divided into those remaining unpeeled, those peeled having visibly broken embryos, and those peeled having unbroken embryos. The latter condition was verified under 40-fold magnification, which also revealed that many peeled seeds with unbroken embryos showed abrasion of the testa. No determination was made of the proportion of these abraded testas that were actually pierced, but the germination percentages in Table 3 would indicate that about half were pierced, since they germinated from 46 to 57%. Indeed, the condition desired from treatment of the utricles is peeling together with testa piercing but without damage to the embryos. Utricles remaining unpeeled after processing germinate no better than

Table 3. Effect of processing utricles using compressed air to strike fruits against metal surface of scarifier.

Condition after processing	% germination after 30 days	Calculated % germination for total sample
Sample A[1] (no processing 100% unpeeled)	2.0	2.0
Sample B		–
7.4% peeled, embryo unbroken	46.0	
4.1% peeled, embryo broken	–	
88.5% unpeeled	–	
Sample C		9.3
9.9% peeled, embryo unbroken	57.3	
12.3% peeled, embryo broken	21.5	
77.8% unpeeled	1.2	
Sample D		6.2
6.8% peeled, embryo unbroken	56.0	
24.5% peeled, embryo broken	6.5	
68.7% unpeeled	1.2	

[1] 3000 utricles per sample.

the untreated, and broken embryos reduce germination. These facts account for the low germination of the total sample.

The intensity of processing was increased progressively from sample B to C to D and this was evidenced by the proportion of each sample that was peeled (11.5%, 22.2%, and 31.3%, respectively). We considered the proportion peeled to be a better measure of treatment severity than the length of time in the scarifier, although these are related. In samples B, C, and D the proportion of the peeled seeds that showed damaged embryos was 35%, 55%, and 78%, respectively. For these samples a straight linear relationship existed between the percent of a seedlot that was peeled and the amount of damage in that portion that was peeled. This is to be expected when seeds once peeled remain in the churning mass of utricles during processing. Were the compressed air stopped periodically and the peeled seeds removed before resuming treatment on the remainder, the damage should be reduced and the yield of peeled seeds increased. This was demonstrated in each of four runs of 390 seconds duration but interrupted every 30 seconds to remove the peeled seeds by screening. Of the 3000 utricles in each run, an average of 75% were peeled using this technique.

The information in the previous two paragraphs is presented not as a fully developed procedure, but rather to be suggestive of an approach which may be applied to utricles of Sereno saltbush before sowing and possibly to other species with small but heavily indurated fruits.

Literature Cited

Arentsen, J. 1972. Absorción foliar de humedad atmosférica y relaciones hídricas en *Atriplex semibaccata* R. Br. y *Atriplex repanda* Phil. Tesis de grado para optar al título de Ingeniero Agrónomo. Escuela de Agronomía de la Universidad de Chile. Santiago. 80 p.

Cristi, A., and J. Gastó. 1971. Alteraciones ambientales y del fruto en la germinación de *Atriplex repanda* Phil. Boletín Técnico 34. Univ. de Chile Facultad de Agronomía, Estación Experimental Agronómica. 40 p.

Graves, W., B. L. Kay, and W. A. Williams. 1974. Seed-treatment studies of seven Mohave Desert shrub species. *In:* Test of seeds of Mohave Desert shrubs. Progr. Rep., Dep. Agron. and Range Sci., Univ. of Calif., Davis. Mimeo 34 p.

Jones, L. G., and R. C. Cobb. 1963. A technique for increasing the speed of laboratory germination testing. Proc. Assoc. Offic. Seed Analysts. 53:144-160.

Laude, H. M. 1951. Treatments to improve the emergence and stand of Smilo grass. J. Range Manage. 4:88-92.

Leighton, G. 1972. Siembra y germinación de *Atriplex repanda* Phil. y otras quenopodiáceas forrajeras en secano y en ambientes controlados. Tesis de grado para optar al título de Ingeniero Agrónomo de la Univ. Católica de Valparaiso. 60 p.

Springfield, H. W. 1970. Germination and establishment of fourwing saltbush in the Southwest. U.S. Dep. Agr. Forest Serv. Res. Pap. RM-55. Rocky Mtn. Forest and Range Exp. Sta., Fort Collins, Colo. 48 p.