

Optimization of *Waltheria indica* Seed Dormancy Relief Treatments and Seed Storage Parameters

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Abstract. In Hawaii, *Waltheria indica* (uhaloa) has been identified for expanded usage as a roadside groundcover in lowland dry ecosystems. Seed dormancy through lack of germination of viable seeds was identified in uhaloa. The presence of physical dormancy in uhaloa seeds was determined and dormancy relief methods were evaluated including hand scarification, dry heat temperature exposure, hot water exposure, and mechanical abrasion in an electric drum scarifier. As a compliment to dormancy relief, long-term storage parameters were evaluated for scarified and nonscarified seeds. The elucidation of physical dormancy was determined through hand scarification, resulting in 96% germination compared with 8% of nonscarified seeds, but is not practical on a large-scale basis. The greatest practical dormancy relief was achieved with a mechanical electric drum scarifier lined with 80-grit sandpaper for a duration of 15 or 30 seconds producing 95% and 99% germination, respectively. Seeds immersed in boiling water for 3 and 5 seconds resulted in 58.6% and 57.7% germination, respectively. Dormancy relief through dry heat exposure was inferior to other relief methods, producing 39% germination at 75 °C for 60 minutes. Nonscarified seeds exhibited minimal loss of viability during 10 months of storage at 5 °C at 12% and 50% relative humidity (RH), but a significant decline in viability of scarified seeds was detected.

Plants produce seeds to ensure the greatest establishment and survival success for future generations (Stoehr and El-Kassaby, 2011). One innate mechanism to increase seedling success is seed dormancy. The critical function of dormancy is to prevent germination when conditions are suitable but when the probability of survival and growth of the seedling is low (Fenner and Thompson, 2005). Generally accepted classification systems define five classes of seed dormancy: physiological dormancy (PD), morphological dormancy, morphophysiological dormancy (MPD), physical dormancy (PY), and combinational dormancy (PY + PD) (Baskin and Baskin, 2004).

Uhaloa [*Waltheria indica* L. (Malvaceae)] is a pantropical shrub species which occurs in diverse populations in the Americas, Mexico, and Brazil (Wagner et al., 1990). Uhaloa in Hawaii is widely classified as a native plant, postulating that the small seeds may have attached to birds that distributed the species to the archipelago (Wester, 1992). Hawaiian uhaloa has been reported in

regions receiving an annual rainfall around 500 mm with distribution ranging from sea level to 1220 m, commonly occurring in altered sites where soil disturbance has occurred (Long and Lakela, 1971; St John, 1979). Uhaloa form is generally described with a single strong stem that frequently branches near the ground (Howard, 1988). In Hawaii, segregation of upright and prostrate growth forms have been observed in locally sourced seedling populations. Axillary inflorescences are usually dense glomeruli that contain fragrant, yellow to orange flowers. Each 2-mm capsule holds one small, black, obovoid seed (Howard, 1974).

A single report on seed germination of uhaloa indicated 13% germination on freshly harvested seed after 16 weeks in a moist germination setting (Sánchez and Uranga, 1993). According to Baskin (2003), water-impermeable seeds (physical dormancy) commonly occur in the Malvaceae family, uhaloa is a member of this plant family (Baskin, 2003). In a study conducted by Boyd, it was determined that in *Fremontodendron decumbens* (also a member of the Malvaceae) 97.8% of seeds were dormant due to an impermeable seedcoat. Breaking of the seedcoat, mechanically or by heat allowed for germination 18–26 times greater than with the nontreated seeds (Boyd and Serafini, 1992). Physical dormancy has been well documented in the native Hawaiian plant *Dodonaea viscosa*, having similarities to the water-impermeable seedcoat of uhaloa and various physical treatments were tested

to break dormancy in seeds of this species (Baskin et al., 2004). One treatment involved mechanically scarifying seeds, after 2 weeks of incubation; seeds that were mechanically scarified had germinated to 96% to 100% in light (Baskin et al., 2004). Nonscarified seeds, on the other hand, germinated to only 0% and 1% in light and darkness, respectively (Baskin et al., 2004). Other methods use to scarified seeds involve exposure to dry heat at a range of 80 to 160 °C, emersion in boiling water and exposing seeds to low RH conditions (Baskin et al., 2004).

Baskin suggests that storage can be used as a dormancy-breaking treatment, but it can also play a fundamental role in optimizing seed longevity. To maximize the usefulness of seeds, it is necessary to understand how possible dormancy relief conditions and long-term storage potential can be achieved through specified storage parameters. Ultimately, seed survival through storage is directly related to the time the seed has been exposed to unfavorable conditions of temperature or humidity (Barton, 1961).

The impact of storage conditions on non-scarified PY dormant *Cassia angustifolia* seed was determined for four levels of RH (5.5%, 11%, 33%, and 75%), established and maintained using saturated salt solutions in airtight desiccators, and three storage temperatures (5 °C, 20 °C, and ambient). Seed viability for *C. angustifolia* was optimized at storage temperatures of 5 and 20 °C when maintained at RH levels of 5.5% and 11%, increased levels of RH reduced seed viability over storage time (Santhoshkumar and Veena, 2012). Baldos et al. (2014) characterized storage and after ripening parameters of the native Hawaiian grass *Heteropogon contortus*. Optimal dormancy relieving conditions for *H. contortus* required a 28- to 30-d equilibration period in a 12% RH desiccation chamber to bring seed moisture levels to 6% followed by a 30 °C storage temperature for 9 to 12 months (Baldos et al., 2014). Although this study was conducted on a grass species with a permeable seed testa, the factors of RH and temperature over time can be applicable for PY dormant seeds which have been scarified before storage.

The assessment of water imbibition can be used to elucidate physical dormancy in seeds (Rolston, 1978). The objectives of this study were to 1) evaluate physical dormancy breaking mechanisms of uhaloa seeds by evaluating seed germination in response to manual and mechanical scarification and exposure to dry heat and hot water emersion and 2) determine the impact of storage humidity and duration at 5 °C on viability and germination response of nonscarified and scarified uhaloa seeds.

Materials and Methods

Plant material. Uhaloa seeds used in this study were derived from two separate harvests/seed batches and represented in two repeated runs of the experiments conducted.

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Seed batch one (SB1) was harvested on the Island of Molokai by the U.S. Department of Agriculture (USDA), Natural Resource Conservation Service, Hoolehua Plant materials center in July 2012 (Sakamoto, personal communication). Seed batch two (SB2) was harvested from the same USDA facility in Mar. 2014. Postharvest handling of both seed batches consisted of air drying, packaging, and 5 °C refrigeration until use (Duvachelle, personal communication). All experiments were initiated first with SB1 between the dates Sept. 2013 to Apr. 2014. SB2 experimentation was initiated between the dates of Apr. to May 2014.

For all dormancy relief studies, experimental units consisted of 50 seeds exposed to the experimental treatment/treatment combinations with four replications, repeated for each seed batch. When statistically significant results were detected, means were separated using Tukey's honestly significant difference test at $\alpha = 0.05$. Each experimental unit was incubated in 90-mm petri dishes premoistened with 3-mL distilled water and lined with filter paper (Whatman no. 2, Little Chalfont, Buckinghamshire). Petri dishes were placed in an alternating temperature germination chamber with four T5 high-output 24-W 6400-K AgroBrite bulbs (Hydrofarm, Petaluma, CA) for 14 h of light at 28 °C and 10 h of dark at 24 °C. Experimental conditions of temperature and RH during the seed germination period were monitored with a Hobo UX100 logger (Onset, Cape Cod, MA). Distilled water was added to petri dishes as needed over the 10-d germination period. Germination was recorded when the seed radicle protruded 1 mm from the seed testa.

Seed batch viability testing. In both seed batches (SB1 and SB2) experimental units were sampled from the larger stock batches following standardized sampling procedures (Elias et al., 2012). Seeds were subjected to viability testing using standard 1% tetrazolium chloride method (Elias et al., 2012; ISTA, 2003). Data were analyzed as a random complete block design in the statistical program JMP Pro 11 (SAS Institute Inc., Cary, NC).

Dormancy relief using hand cut mechanical scarification. Uhaloa seeds were hand scarified using a scalpel, with visual guidance provided with $\times 20$ magnification under a dissection microscope to remove a small piece of testa exposing unaffected endosperm. The experimental units for both seed batches were sampled following techniques outlined by Elias et al. (2012). Data were analyzed as a split-plot design in the statistical program JMP Pro 11. The main plot effect was seed batch, and the split-plot effect was hand scarification (Jones and Nachtsheim, 2009).

Dormancy relief through exposure to dry heat. Seed germination response to dry heat temperatures of 50, 75, 100, and 125 °C, in a Quincy laboratory model 40GC laboratory oven (Quincy Laboratory Inc., Chicago, IL) and exposure times (0, 1, 5, 15, 30, and

60 min) were determined. During the seed exposure time, oven temperature was manually recorded with a thermometer. After each exposure interval, seeds were allowed to cool to room temperature for 1 min before the start of the seed germination phase of the experiment. Data were analyzed as a split-split-plot design in the statistical program JMP Pro 11. The main plot effect was seed batch, the split-plot effect was oven temperature and the split-split-plot effect was duration exposed to treatment temperatures.

Dormancy relief effect through exposure to boiling water. The relief of physical dormancy was evaluated by exposing uhaloa seeds to a range of boiling water emersion times. Seed experimental units were dipped in boiling distilled water for 0, 1, 3, 5, 10, 15, 30, or 60 s. All experimental treatments and the control were dipped in distilled ice water for 5 s to cool the seeds and placed in a petri dish lined with filter paper with 3 mL of distilled water. The hot water bath temperature was measured to quantify temperatures and a stir bar was used to ensure a homogeneous heated water solution. The water temperature was held constant at 101 °C throughout the entire experiment and the cold water bath ranged from 12.5 to 14.0 °C. Seeds that did not germinate were visually inspected to determine if water imbibition occurred and for the presence of seed deterioration through rotting. Data were analyzed as a split-plot design in the statistical program JMP Pro 11. The main plot effect was seed batch and the split-plot effect was the duration of seed exposure to boiling water.

Dormancy relief using a mechanical sandpaper drum scarifier. Seed scarification was evaluated using a commercially available mechanical sandpaper scarifier to relieve physical dormancy in uhaloa seeds. Scarification was conducted in a Forsberg seed scarifier with a 1/3-horsepower electric motor (Forsberg, INC., Thief River Falls, MN). Sandpaper coarseness is classified with a Coated Abrasive Manufacturers Institute grit designation, the 80, 60, and 40 grits used have average particle diameters of 190, 265, and 425 μm , respectively. The experiment evaluated three grits of sandpaper (40, 60, and 80 grit) at three exposure times of 15, 30, and 45 s. Data were analyzed as a split-split-plot design in the statistical program JMP Pro 11. The split-split-plot model was designed with seed batch as the main plot effect, sandpaper coarseness as the split-plot effect and duration in the scarifier as the split-split-plot effect.

Impact of storage conditions on seed viability of scarified and nonscarified seed to evaluate seed preservation. Both scarified and nonscarified seeds were partitioned into 0.25 g (≈ 200 seeds) units and placed in open 1.5-mL microcentrifuge tubes with O-ring gasket lid (Fisher Scientific, Pittsburgh, PA). Scarified seeds were mechanically treated in a Forsberg drum scarifier lined with 80-grit sandpaper operating for a duration of 30 s. Since the seed testa of PY seed is

impermeable to water vapor, the nonscarified treatments represented a control. Seeded tubes were equilibrated at two RH levels inside sealed Bel-Art (Scienceware, Wayne, NJ) nonvacuum desiccation chambers. Humidity levels were set and maintained using saturated salt solutions of lithium chloride (12% RH) and calcium nitrate (50% RH) (Baldos et al., 2014; Greenspan, 1977). After the 28-d humidity equilibrium period, seed sample tubes were sealed and transferred to a 5 °C storage temperature. Seeds were withdrawn from the temperature chambers at intervals of 2 months with the last sample removed after 10 months. Seeds were evaluated for viability (Elias et al., 2012) and germination percentages at each time interval. Data were analyzed as a split-split-split plot using the general analysis of variance fit model function in the statistical software program Statistix 10.0 (Analytical Software, Tallahassee, FL). In the split-split-split-plot model statement, seed batch was the main plot effect, scarification the split-plot effect, storage humidity the split-split-plot effect, and months of storage as the split-split-split-plot effect. Treatment response data included seed viability and percent germination. Nonscarified seeds were evaluated for germination at each storage interval to determine if dry storage alone could impact the physical dormancy imposed by the seedcoat.

Results

Seed batch viability testing. Tetrazolium testing for seed viability indicated that 96.3% of seeds sampled from SB1 were viable with a significantly lower level of 62.5% recorded for SB2. Viability values for both seed batches will be used to standardize germination results in subsequent dormancy trials using the equation:

$$\text{Percent standardized germination} = \frac{\text{Percent observed germination}}{\text{Percent viable seeds}} \times 100$$

Dormancy relief through hand cut mechanical scarification. There was no interaction between the factors of seed batches and cutting treatment ($P = 0.74$), allowing for the pooling of treatment means across the factor of seed batch (Gomez and Gomez, 1984). Hand cut treatments resulted in an average germination of 96% compared with 8% of nonscarified treatments ($P < 0.001$).

Dormancy relief effect through exposure to dry heat. There was no significant interaction between the factors of seed batch \times temperature \times duration ($P = 0.0718$); however, a significant interaction between temperature \times duration ($P = <0.0001$) was detected. The treatment that resulted in the significantly highest germination was exposed to a temperature of 75 °C for 60 min, with a mean germination of 39.4%. Treatments exposed to 75 °C for 30 and 15 min were significantly lower than the 60-min treatment but provided the second and

Table 1. Germination means (%) of uhaloa seeds exposed to four dry heat treatments over six durations.

| Temperature (°C) | Duration (min) ^{z,y} | | | | | |
|------------------|-------------------------------|---------|--------|---------|--------|--------|
| | 0 | 1 | 5 | 15 | 30 | 60 |
| 50 | 8.6 d | 17.0 c | 20.0 c | 18.7 c | 18.2 c | 18.4 c |
| 75 | 9.5 d | 19.2 c | 20.0 c | 23.7 bc | 27.7 b | 39.4 a |
| 100 | 8.4 d | 22.0 bc | 19.0 c | 9.5 d | 0.9 e | 0.0 e |
| 125 | 9.5 d | 18.8 c | 0.3 e | 0.0 e | 0.0 e | 0.0 e |

^zMeans within columns and rows followed by the same letter are not significantly different according to Tukey's honestly significant difference comparison at $P \leq 0.05$.

^yMeans are reported from four experimental replications.

Table 2. Germination response means (%) of uhaloa seeds exposed to 101 °C water emersion over eight durations.

| Duration (s) | Germination (%) ^{z,y} |
|--------------|--------------------------------|
| 0 | 8.8 e |
| 1 | 37.3 d |
| 3 | 58.6 a |
| 5 | 57.7 ab |
| 10 | 50.2 bc |
| 15 | 46.5 c |
| 30 | 43.7 cd |
| 60 | 38.8 d |

^zMeans followed by the same letter are not significantly different according to Tukey's honestly significant difference comparison at $P \leq 0.05$.

^yMeans are reported from four experimental replications.

third numerically highest germination. Minor stimulation in germination was recorded in treatments exposed to 50 °C. The 75 °C treatments exhibited a general positive linear trend until the maximum germination treatment. Nongerminated seeds exposed to low temperature treatments (50 and 75 °C) remained viable, with no disruption to the mechanism of PY. Temperature exposure of 100 °C resulted in stimulation at 1 min of exposure, then decreased steadily to zero germination at 30 min of exposure. A similar effect occurred at 125 °C in which stimulation occurred at 1 min of exposure then rapidly decreased to zero germination at 5 min (Table 1).

Dormancy relief effect through exposure to boiling water. There was no significant interaction detected between the factors of seed batch × treatment ($P = 0.7719$), therefore treatment means will be pooled for the factor of seed batch. All boiling treatments resulted in significantly greater germination than the untreated seeds. Optimal germination was found at a boiling duration of 3 and 5 s, with 58.6% and 57.7% germination, respectively. Germination decreased as exposure times increased until the final 60-s duration, resulting in 38.8% germination (Table 2). The reduced germination and subsequent viability loss at longer exposure durations was attributed to seed death due to overheating and was confirmed visually by the presence water saturated rotting endosperms.

Dormancy relief using mechanical sandpaper drum scarifier. There was a significant interaction between the factors of seed batch × sandpaper × exposure time ($P = 0.001$), thus the results will be presented for each seed batch. In both seed batches, the highest level of germination occurred with

80-grit sandpaper exposed at 15 and 30 s, although the 30-s treatment in both seed batches was numerically optimal. As exposure duration increased, especially as sandpaper grit decreased (more coarse particles) seeds were pulverized causing endosperm and embryo damage, lowering germination and viability rates. The decrease in germination due to damage was more pronounced in SB2 compared with SB1 (Table 3).

Impact of storage conditions on seed viability of scarified and nonscarified seed to evaluate seed preservation. A significant interaction was detected between the factors of seed batch × scarification × month, therefore results are presented for each seed batch ($P = 0.032$), pooled over both humidity levels. Within SB1 the highest seed viability was found at month 0 (start of trial) in both the scarified and nonscarified treatments (95% and 98%, respectively). In nonscarified treatments, viability decreased to 92% at month 10. Scarification significantly reduced viability after month 2 of storage until month 10 (92% to 71%, respectively) (Table 4). In SB2, there was no viability loss with storage of nonscarified seeds. Scarified seeds from SB2 followed a similar pattern of viability loss as seeds from SB1 (i.e., ≈25% loss in viability with scarification and 10 months of storage).

In SB2, the highest seed viability was recorded at month 0 in the scarified treatment (65%). Seed viability remained stable throughout the 10 months of storage for the nonscarified seeds (Table 4). Scarified seeds lost viability significantly with time from month 2 until month 10 (58% to 40%, respectively). The decline recorded in viability in the scarified treatments over the durations in both seed batches indicate that scarification before storage is not recommended. Humidity levels evaluated during storage did not have a significant effect on seed viability. Since viability in nonscarified seeds

remained constant during storage, germination was analyzed to determine if dormancy relief was occurring over storage duration. The analysis indicated that there was no significant interaction between the factors of seed batch × months ($P = 0.199$), therefore results will be pooled over seed batch. Analysis of germination data of nonscarified seeds indicated a slight reduction from month 0 until month 10 (7.5% to 5%, respectively). Nonscarified seeds are not relieved of dormancy over storage durations up to 10 months (Table 5).

Discussion

Seed viability was seen to have differed significantly between the two seed batches used throughout the range of experiments conducted. Uhaloa seed harvested by the USDA Hoolehua Plant Materials Center in 2012 (SB1) was considered "late" (Sakamoto, personal communication), as a result seeds were mostly mature and shattering from the mother plants. For the 2014 seed batch (SB2), harvest timing was considered earlier than the 2012 harvest time resulting in a larger percentage of immature seed with potentially thinner seedcoats. The 2012 harvest yielded 1.8 kg of seed compared with the 2014 harvest of 11.3 kg, from the same field with ≈800 harvested plants. The harvester combine (Massey Ferguson MF-17/19; Kincaid Equipment Manufacturing, Haven, KS) used to collect seeds from the field, used settings that changed between the 2012 and 2014 harvests. For the 2014 seed harvest, the alteration reduced the gap between the thresher wheel and the concave of the combine, causing more mechanical damage to the seeds harvested (Duvachelle, personal communication). The reduced viability in the 2014 seed batch was attributed to the combination of the greater percentage of immature seeds and more physical damage during harvesting.

Dormancy was determined to be physically imposed based on the results of the hand cutting of uhaloa seeds; however, the time to hand scarify 200 seeds is not practical for any large-scale seed processing. Optimization of the dormancy relief methods proved to be most effective and efficient in both seed batches using an electric drum sandpaper scarifier with 80-grit sandpaper for a duration of 30 s. Using sandpaper for seed scarification to relieve physical dormancy is well

Table 3. Germination means (%) for two seed batches (SB1 and SB2) of uhaloa seeds exposed to mechanical abrasion in a sandpaper scarifier with three levels of sandpaper grit and four exposure durations.

| Treatment sandpaper (grit) | Duration (s) ^{z,y} | | | | | | | |
|----------------------------|-----------------------------|--------|--------|--------|--------|---------|---------|----------|
| | 0 | | 15 | | 30 | | 45 | |
| | SB1 | SB2 | SB1 | SB2 | SB1 | SB2 | SB1 | SB2 |
| 40 | 13.5 g | 12.8 g | 79.4 b | 77.1 | 74.3 b | 29.6 de | 27.5 ef | 16.0 fg |
| 60 | 14.0 g | 9.9 g | 76.4 b | 80.4 b | 81.5 b | 40.8 cd | 45.7 c | 17.6 efg |
| 80 | 13.5 g | 9.7 g | 95.2 a | 94.5 a | 97.1 a | 98.4 a | 71.7 b | 42.4 c |

^zMeans within rows and columns followed by the same letter are not significantly different according to Tukey's honestly significant difference comparison at $P \leq 0.05$.

^yMeans are reported from four experimental replications.

Table 4. Seed viability means (%) of scarified and nonscarified uhaloa seeds over 10 month of storage at 5 °C, represented for seed batch one and two.

| Seed treatment | Months of storage (seed batch 1) ^{z,y} | | | | | |
|----------------|---|---------|---------|---------|---------|---------|
| | 0 | 2 | 4 | 6 | 8 | 10 |
| Nonscarified | 97.8 a | 95.4 ab | 93.3 ab | 94.3 ab | 93.1 ab | 92.4 bc |
| Scarified | 95.4 ab | 91.5 bc | 87.1 c | 80.6 d | 77.1 d | 70.5 e |

| Seed treatment | Months of storage (seed batch 2) | | | | | |
|----------------|----------------------------------|---------|---------|---------|---------|---------|
| | 0 | 2 | 4 | 6 | 8 | 10 |
| Nonscarified | 62.1 fg | 62.9 fg | 61.4 fg | 61.3 fg | 61.7 fg | 61.5 fg |
| Scarified | 64.8 f | 57.9 gh | 53.5 hi | 50.6 i | 50.0 i | 39.6 j |

^zMeans within rows and columns followed by the same letter are not significantly different according to Tukey's honestly significant difference comparison at $P \leq 0.05$.

^yMeans are reported from four experimental replications.

Table 5. Germination means (%) for storage potential of nonscarified uhaloa seeds over 10 month, pooled over two seed batches.

| Months | Germination (%) ^{z,y} |
|--------|--------------------------------|
| 0 | 7.5 a |
| 2 | 7.0 ab |
| 4 | 5.5 ab |
| 6 | 5.8 ab |
| 8 | 5.6 ab |
| 10 | 5.0 b |

^zMeans followed by the same letter are not significantly different according to Tukey's honestly significant difference comparison at $P \leq 0.05$.

^yMeans are reported from four experimental replications.

established (Egley, 1979; Hutchison and Ashton, 1979), but customizing the technique to a species-specific regime is imperative (Olszewski et al., 2010).

Storage parameters evaluating the effects of seeds scarified and nonscarified over a 10-month duration was designed to streamline the process of scarification and storage. The intent of this experiment was to enable to end user of the seeds a more streamline process, where seed could be immediately ready for use on removal from storage. However, the decline in seed viability over 10 months with scarified seeds indicates this procedure is not recommended for long-term storage after 2 months. On the basis of treatments evaluated, viability of uhaloa seed is maintained at high levels when nonscarified seeds are stored at 5 °C at both 12% and 50% equilibrated RH for up to 10 months. The duration of storage of SB1 at 5 °C before initiation of experimentation further indicates the ability for nonscarified seeds

to be stored for extended periods without a significant loss of viability.

The research presented here supports the following protocols for the utilization of uhaloa from seed stock. If seeds are not needed immediately after harvest, store at 5 °C at either 12% or 50% RH. When seeds are desired for use, scarify using an electric drum scarifier with 80-grit sandpaper for a duration of 30 s to relieve physical dormancy. Once the seeds are scarified, viability will decline significantly after 2 months of storage at low temperatures.

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