Torrid Panicgrass Dormancy Relief Through Exogenous Chemical Treatments and Seed Storage Optimization of Temperature and Relative Humidity Parameters

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ADDITIONAL INDEX WORDS. germination, Hawaii, native, *Panicum torridum*, physiological dormancy, seed viability

SUMMARY. Seed dormancy is an evolutionary adaptation for increasing seedling survival by delaying germination and is found in many families of seed plants. Although dormancy is ecologically important, it becomes problematic during agronomic production and restoration. Torrid panicgrass (*Panicum torridum*) is a native Hawaiian annual grass that has been identified as a re-vegetation candidate for seasonally dry areas. Torrid panicgrass seed appears to possess a nondeep to intermediate physiological dormancy. This research aimed to characterize dormancy relief parameters by 1) evaluating exogenous hormonal, reactive oxygen intermediates, and simulated combustion product treatments; and 2) determining optimized storage conditions of relative humidity (RH) and temperature over a 10-month duration. Results indicate that all exogenous chemical treatments tested were not effective at relieving the dormancy present in torrid panicgrass. Optimal storage conditions to relieve dormancy were found with seeds equilibrated to 12% RH, stored at 30 °C for a period of 8 months resulting in 55% germination. Maintenance of viability for long-term storage up to 10 months was best achieved with seeds stored at 12% RH at 10, 20, or 30 °C.

Seed dormancy is an evolutionary adaptation for increasing seedling survival by delaying germination until conditions are favorable for seedling establishment (Fenner and Thompson, 2005). Although seed dormancy is ecologically important, it imposes hardships during agronomic production and restoration. Classification systems define five classes of seed dormancy: physiological dormancy (PD), morphological dormancy (MPD), morphophysiological dormancy (MPD), physical dormancy (PY), and combinational dormancy (PY + PD) (Baskin and Baskin,

2004). Within the PD class, three levels can be defined as nondeep, intermediate, and deep, which can be partially elucidated by germination response to stimulatory chemicals (Baskin and Baskin, 2004). In many species, the depth of seed dormancy is controlled by the relationship between levels of gibberellic acid (GA) and abscisic acid (ABA) and is generally referred to as the hormone-balance theory (Wareing and Saunders, 1971). This theory proposes that as dormant seeds reach the optimum ratio of endogenous GA: ABA, germination can proceed. This is the foundation for the widely accepted practice of treating dormant seeds with GA₃, a well-established dormancy-breaking chemical (Kirmizi

et al., 2010), and GA₄₊₇, which is almost 1000 times more effective than GA₃ (Hilhorst, 2011). Germination stimulation by GA is limited to the nondeep and intermediate levels of PD (Baskin and Baskin, 2004). Another method to induce germination involves the use of fluridone, which decreases the levels of endogenous ABA during early stages of imbibition (Grappin et al., 2000). Ethylene, which is also a hormonal treatment, has been recognized to relieve dormancy in seeds. It is produced in trace amounts by almost all higher plants and is involved in the control of growth and development processes that range from germination to senescence (Abeles et al., 2012). Exogenous ethylene stimulates the germination of dormant and nondormant seeds, although in some cases seed germination is unaffected by this hormone (Kępczyński and Kępczyńska, 1997). Ethephon, an ethylene-releasing compound, has been found to break dormancy more efficiently compared with ethylene (a gas at room temperatures). Aside from hormonal treatments, reactive oxygen donors such as sodium nitroprusside (SNP) and hydrogen peroxide can enhance germination or relieve dormancy in seeds (Beligni and Lamatina, 2000; Bethke et al., 2011; Sarath and Mitchell, 2008). For species that are fire adapted, such as the native Hawaiian grass species tanglehead (*Heteropogon contortus*), simulated combustion products liquid smoke (a commercial food-grade flavoring) and cyanide can be effective germination promoters (Baldos, 2013).

Because seeds exhibiting intermediate or deep PD are not generally affected by stimulatory dormancy relief chemicals, it is necessary to understand optimum after-ripening storage conditions to maximize seed germination (Baskin and Baskin, 2004). Ultimate seed survival is

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
25.4	inch(es)	mm	0.0394
16.3871	inch ³	cm ³	0.0610
28.3495	oz	g	0.0353
28,350	oz	mg	3.5274×10^{-5}
1	ppm	mg·L ⁻¹	1
(°F - 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32
(°F ÷ 1.8) + 255.37	°F	K	(K - 255.37) × 1.8

directly related to the time the seed has been exposed to unfavorable conditions of temperature or humidity (Barton, 1961). Numerous studies have used sealed chambers to evaluate the factors of temperature and RH to achieve dormancy relief (Baldos et al., 2014; Santhoshkumar and Veena, 2012). When using sealed desiccation chambers, specific levels of RH can be established and maintained using saturated salt solutions (Winston and Bates, 1960; Young, 1967), but proportions of water added to the salt (forming the saturated salt solution) to desiccate a given volume of water (contained in fresh seeds) are unclear.

Torrid panicgrass is a native Hawaiian annual grass that has been identified as a re-vegetation candidate for roadsides and conservation plantings in Hawaii. The intent of this study was to evaluate seed dormancy relief in torrid panicgrass through 1) exposure to the exogenous chemicals cyanide, ethanol, GA₃, GA₄₊₇, liquid smoke, hydrogen peroxide, fluridone, ethephon, and sodium nitroprusside; and 2) storage conditions equilibrated to three levels of RH, stored at three temperatures over 10 months. The treatments of desiccation were chosen to resemble the natural ecological exposure of torrid panicgrass in the wild. Before the dormancy relief storage study, a calibration procedure was used to determine the components of the saturated salt lithium chloride (LiCl) solution that could absorb moisture released by seeds in a sealed vessel while lowering and maintaining the target RH level.

Materials and methods

PLANT SEED MATERIAL. Two separate batches of torrid panicgrass seeds were used throughout these experiments to determine the consistency of the treatment response in replicated trials. Both seed batches were harvested from wild populations found on the Hawaiian island of Molokai and stored in sealed bags in a 10 °C refrigerator until use. Seed batch 1 (SB1) was harvested in May 2013. Seed batch 2 (SB2) was harvested in May 2014. Before experimental use, seeds were air dried and stored in sealed containers at 5 °C. Both seed batches showed higher than 90% seed viability before experimental evaluation using a 1% tetrazolium chloride (TZ) solution following

standardized methods outlined by the International Seed Testing Association (1996).

GERMINATION PARAMETERS. For all germination studies, experimental units consisted of 50 seeds exposed to the experimental treatment/treatment combinations with four replications, repeated for each seed batch. Each experimental unit was incubated in 90-mm petri dishes premoistened with 3 mL distilled water and lined with filter paper (Whatman #2; GE Healthcare, Little Chalfont, UK). Petri dishes were placed in an alternating temperature germination chamber with four T5 high output 24-W 6400-K bulbs (AgroBrite; Hydrofarm, Petaluma, CA) for 14 h of light at 28 °C and 10 h of dark at 24 °C. Experimental conditions during the seed germination period were monitored and recorded with a data logger (Hobo UX100; Onset Computer Corp., Bourne, MA); 1.5 mL of distilled water was added to petri dishes as the perimeter of the filter paper began to appear dry over the 14-d germination period. Germination was recorded when the seed radicle protruded 1 mm from the seed testa. Seed viability testing was not conducted on the remaining seeds that did not germinate after the 14-d period.

WATER IMBIBITION OF SEED. To help classify dormancy type, it was necessary to determine if the seed testa was permitting water to pass through to the seed endosperm. Although it is well established that seed-coats of grasses are water permeable, this study attempted to provide a framework of methods for evaluating dormancy. This study evaluated the ability of after-ripened germinable torrid panicgrass seeds to imbibe water compared with freshly harvested nongerminable seeds. When fresh seeds are able to imbibe water and not germinate, it is unlikely that a physical dormancy mechanism is prohibiting germination. Weight increases were measured in 100 seed units over a 24-h period exposed to distilled water on filter paper, with three replications. Weight measurements were taken before exposure to water, and after exposure at 1, 2, 4, 12, and 24 h. At each interval, seeds were removed from the saturated filter paper, blotted dry, weighed, and returned to the filter paper

(Baskin et al., 2004). Weight was measured using an analytical balance with a weight sensitivity range of 0.1 mg. The amount of water taken up was determined by actual weight increases and converted to the percentage weight increase:

$$Wi = \frac{(Wi - Wd)}{Wd} \times 100,$$

where *Wi* and *Wd* are weight of imbibed and dry seeds, respectively.

A random sample of the germinable seed batch used for the imbibition study had a 55% germination at the start of the experiment, with high viability (90%). The freshly harvested seed was of high viability (96%) but fully dormant with no germination. Data expressed as percent seed moisture increase was compared between germinable torrid panicgrass seed and freshly harvested seed over imbibition exposure times. Data were analyzed for weight increases as a split-plot with seed batch (i.e., germinable and fresh) as the main effect and imbibition time as the split-plot effect, in the statistical software program Statistix (version 10.0; Analytical Software, Tallahassee, FL).

DORMANCY RELIEF THROUGH EXOGENOUS CHEMICALS. After determining that freshly harvested seed could not germinate, seed response to known germination stimulators was determined to characterize the physiological nature of torrid panicgrass dormancy. Torrid panicgrass seeds were exposed to treatments of cyanide, ethanol, GA₃, GA₄₊₇, liquid smoke, hydrogen peroxide, fluridone, ethephon, and sodium nitroprusside individually to determine their effect on dormancy relief (Table 1). Exposure was achieved by making solutions of the desired chemicals with distilled water, then applying aliquots directly to the petri dish with the dormant seeds. Germination responses of zero to less than 4% were excluded from the analysis due to their impact on the magnitude of mean square error and the potential to magnify treatment effects (Reeve and Strom, 2004). Data were analyzed as a random complete block design using standard least squares fit model in the statistical program JMP Pro (version 11; SAS Institute, Cary, NC).

DESICCATION CHAMBER CALIBRATION. Twenty grams of LiCl salt was mixed with 0, 3.0, 6.0, 12.0 and 24.0 mL of distilled water. These

Table 1. List of exogenous chemicals, rates, and respective citations tested to relieve dormancy in torrid panicgrass seeds. Exposure was achieved by making solutions of the desired chemicals with distilled water, then applying aliquots directly to the petri dish with the dormant seeds.

Treatment	Rate ²	Citation
Ethanol	0.3 M	Tischler and Young (1983)
Hydrogen peroxide	20 mM	Sarath and Mitchell (2008)
Gibberellic acid (GA) GA ₃	100 ppm	Kirmizi et al. (2010)
	200 ppm	
GA ₄₊₇	50 μ M	Hilhorst (2011)
	100 μ M	
	200 μ M	
	400 μ M	
	800 μ M	
Liquid smoke	1%	Baldos (2013)
Potassium cyanide	0.5 mM	Bethke et al. (2011)
Fluridone	10 μ M	Grappin et al. (2000)
	20 μ M	
	40 μ M	
	80 μ M	
Ethephon	0.5 mM	Kećpczyński and Kećpczyńska (1997)
	1.0 mM	
	2.0 mM	
	4.0 mM	
Sodium nitroprusside (SNP)	50 μ M	Bethke et al. (2011)
	100 μ M	
	150 μ M	
	200 μ M	
Distilled water	-	

²1 ppm = 1 mg·L⁻¹.

water/salt mixtures were placed in a sealed desiccation chamber that contained 5 mL of water in a separate container. The drop in RH was recorded inside the chamber for 18 d with a data logger (Hobo UX100) to determine the optimal composition of saturated salt solutions (the proportions of salt and water) and the moisture levels within the desiccation chambers that could be absorbed but still reduce and maintain the reported level of RH.

DORMANCY RELIEF AND VIABILITY MAINTENANCE THROUGH STORAGE CONDITIONS. Seed after-ripening conditions were evaluated for the parameters of temperature and RH for both seed batches. Three RH levels were imposed on seeds placed into unsealed 1.5-mL microcentrifuge tubes with O-ring gasket lids (Fisherbrand; Fisher Scientific, Pittsburgh, PA) and placed in sealed nonvacuum desiccation chambers (Bel-Art; Scienceware, Wayne, NJ). Humidity levels were maintained using saturated salt solutions of LiCl (12% RH), calcium nitrate (50% RH), and sodium chloride (75% RH) (Winston and Bates, 1960; Young, 1967). Each desiccation chamber

contained a petri dish with 20 g of salt mixed with 6 mL of distilled water to reduce and maintain specific levels of RH based on the type of salt used. After 30 d in the sealed desiccator, seed-filled tubes were immediately sealed and transferred to three incubation temperature (10, 20, and 30 °C) chambers. Seeds were withdrawn from the temperature chambers at 0, 2, 4, 6, 8, and 10 months to test for germination, seed viability (TZ), and moisture content. Throughout the experiment, temperature and humidity levels within the chambers were monitored in real time and logged electronically with data loggers (Hobo UX100).

Germination data were analyzed as a split-split-split-split-plot, classifying seed batch at the main-plot effect, RH as sub-plot effect, temperature as sub-sub-plot effect and duration in storage as sub-sub-sub-plot effect in the statistical software program Statistix 10.0. Due to inconsistent germination found in month 0 in SB1, and no germination in SB2, data for month 0 was removed from the analysis. The remaining analysis for germination data was conducted over the three factors at durations of 2, 4, 6, 8,

and 10 months. The analysis for seed viability was conducted with the same data analysis format as germination, but all months of storage were included in the analysis.

Results

WATER IMBIBITION OF SEEDS. No significant interactions were present between the factors of seed batch $\times$ imbibition time on seed weight increases, thus treatment means were pooled over seed batch ($P = 0.057$). The results indicated a significant weight increase over the 24-h imbibition period ($P < 0.001$). Both germinable seeds and freshly harvested seeds were similar in their ability to imbibe water, with both seed batches showing a 15.3% final seed weight increase. Imbibition weights significantly increased within the first 4 h of incubation and leveled off thereafter (Table 2). This result indicates that the torrid panicgrass seed testa is not limiting the uptake of water into the endosperm, an indication that dormancy is not physically based [PY (Baskin et al., 2004)].

DORMANCY RELIEF THROUGH EXOGENOUS CHEMICALS. Torrid panicgrass seeds were not stimulated by any of the exogenous chemicals tested. All treatments and rates resulted in zero germination, despite seeds exhibiting viability. The lack of response may indicate that the dormancy present is a nondeep to intermediate form of PD (Baldos et al., 2015; Baskin and Baskin, 2004).

DESICCATION CHAMBER CALIBRATION. Calibration of saturated salt solutions indicated that 20 g of LiCl combined with 6 mL of distilled water reduced ambient humidity levels (50%) to target 12.5% RH within 6 d in a 1700-cm³ chamber in the presence of 5 mL of distilled water. The 12.5% target RH was not achieved with the other volumes of water added to the 20 g of LiCl (Table 3). These results confirm the effectiveness of the salt and water mixture to remove the required amount of moisture in torrid panicgrass seeds during dry seed storage to provide dormancy relief and viability maintenance. The other salts used in this research (calcium nitrate and sodium chloride) are required to absorb less moisture (to impose higher RH levels of 50% and 75%) from the chambers, therefore the water volumes to produce

Table 2. Torrid panicgrass seed percent weight increase over a 24-h distilled water imbibition period. No interactions between the effects of fresh or germinable seed were detected, thus combined results are presented.

Time (h)	Wt increase (%)
0	0.00 c ^z
1	5.21 b
2	8.14 b
4	15.64 a
12	14.66 a
24	15.31 a

^zMeans are separated using Tukey's honestly significant difference comparison at $P = 0.05$. Means within columns followed by the same letter are not significantly different.

their saturated salt solutions were based on the LiCl calibration.

DORMANCY RELIEF THROUGH STORAGE CONDITIONS. The highest level significant interaction was found among the three factors of temperature $\times$ RH $\times$ months in storage ($P < 0.001$), allowing for means to be pooled across seed batches. The highest level of germination was recorded when storage conditions of 12% humidity at 30 °C were imposed on dormant seeds (Fig. 1). Under these conditions, germination increased steadily up to 55% at 8 months in storage and then dropped slightly to 49% at 10 months (Table 4). Storage at 20 °C (12% RH) also showed similar but consistently lower levels of germination enhancement that peaked at 6 months at 20% and did not significantly change with an additional 4 months of storage. Storage at 10 °C (12% RH) resulted in minimal germination, peaking at less than 10% germination with 8 months of storage. At 75% humidity, there was minor stimulation in germination with storage. The germination response at 50% RH was similar to that at 75% with only slight enhancement during the storage duration when the 30 °C temperature was imposed on dormant seeds.

VIABILITY MAINTENANCE THROUGH STORAGE CONDITIONS. A significant four-way interaction was identified for the factors of seed batch $\times$ temperature $\times$ RH $\times$ months in storage ($P < 0.001$), thus factors of temperature, RH, and storage duration were presented separately for both seed batches. The general trend between both seed batches was that at

Table 3. Desiccation chamber relative humidity (RH) calibration using saturated lithium chloride. The optimal RH level of 12.5% was obtained by adding 6 mL distilled water to 20 g (0.7 oz) lithium chloride in a 1700-cm³ (103.7-inch³) desiccation chamber.

Water added (mL) ^z	RH level achieved (%)	Time to RH equilibration (d)
0	13.0	6
3	13.0	6
6	12.5	6
12	13.5	5
24	26.0	4

^z1 mL = 0.0338 fl oz.

Fig. 1. Seed dormancy relief through germination of torrid panicgrass exposed to varying levels of relative humidity (RH) and temperature over a 10-month storage duration. Data are pooled over two seed batches and presented with SE bars; $1.8 \times ^\circ\text{C} + 32 = ^\circ\text{F}$.

Table 4. Torrid panicgrass percent germination means representing dormancy relief based on varying levels of relative humidity (RH) and temperature over a 10-month storage duration. Shading over values indicates the optimal treatments.

Storage temp (°C) ^z	RH (%)	Seed germination (%)				
		Time in storage (mo.)				
		2	4	6	8	10
10	12	3 v ^y	5 s-v	7 r-v	7 r-u	4 uv
	50	11 l-r	11 l-r	16 g-k	12 j-p	9 n-s
	75	9 n-s	8 n-t	8 p-u	7 q-u	4 t-v
20	12	12 j-p	15 j-l	24 de	24 de	25 de
	50	8 q-u	9 m-s	16 g-j	15 g-l	14 h-l
	75	9 m-r	8 o-t	9 n-s	7 q-u	8 n-t
30	12	19 fg	31 c	48 b	55 a	49 b
	50	13 i-n	18 f-h	27 d	21 ef	17 f-i
	75	9 m-r	16 g-k	12 k-q	13 i-m	12 j-o

^z $1.8 \times ^\circ\text{C} + 32 = ^\circ\text{F}$.

^yMeans are separated using Tukey's honestly significant difference comparison at $P = 0.05$. Means within columns and rows followed by the same letter are not significantly different. Data are pooled over two seed batches.

the lowest RH level (12%), viability loss was minimized over the 10-month duration. As RH level was raised to 50% and 75%, increasing losses of viability were detected over the storage duration, with the lowest

seed viability at 75% RH. Within the 12% RH level, temperature increases produced a slight decline in viability. The 50% RH level exhibited a more defined loss of viability as temperatures increased over time. Within the

75% RH treatments, increased temperatures produced the most distinct and reduced losses of viability, with the greatest viability losses found with the highest temperature of 30 °C (Fig. 2). In SB2, the magnitude of viability loss was greater than SB1 and most obvious at the highest RH (75%) and the warmest temperature (30 °C) over 10 months. Torrid panicgrass seed viability was maintained at the highest levels at 12% RH during the 10 months of storage within the temperature range of 10 to 30 °C. RH levels above 12% reduced seed viability and are not recommended during storage.

Discussion

There was no restriction of water uptake through seed testa to the endosperm, reinforcing the hypothesis that the dormancy present is not physically based. Following the logical progression of treatments to relieve dormancy led to the evaluation of PD with exogenous chemical treatments (Baskin and Baskin, 2004). As noted in the results, no germination stimulation was detected in response to any of the chemicals evaluated. It is well established that different plant

species frequently exhibit significant variations in their germination responses to exogenously imposed chemical stimulants (Bewley, 1997).

Differences detected between the storage response of SB1 and SB2 at the initiation (month 0) could be attributed to the duration that SB1 was stored before use in the experiment. SB1 was stored in a 10 °C refrigerator for 4 months before evaluation of storage variables on dormancy relief, compared with SB2, which was used 2 weeks after harvest. Ideally, studies on factors impacting seed dormancy relief should be conducted on seeds collected from the field as soon after harvest as possible. The wild stands of torrid panicgrass that were the source of seeds for these studies were located in remote areas of Molokai, extending the time between seed collection and the initiation of experiments. It has also been reported that the depth of dormancy can be affected by time of year harvesting of tanglehead seeds (Baldos et al., 2014). Both torrid panicgrass seed batches used in this research were harvested in May but were separated by 1 year, thus the unrecorded environmental conditions at the collection sites could account

for differences in the observed depth of dormancy (Andersson and Milberg, 1998).

When using saturated salts for humidity regulation, it is recommended that salt and water proportions are calibrated for their impact within the chambers used for controlled seed desiccation. This research supports the addition of 6 mL distilled water to 20 g of LiCl to provide the reduction and maintenance of the target 12% RH for 11 g of torrid panicgrass seed in a 1700-cm³ desiccator. Calibration methods discussed in this paper can be up-scaled for seed desiccation in a larger-capacity chamber, provided that the entire volume of seed can be uniformly exposed to the desired level of RH. A practitioner can calculate the amount of saturated salts needed to desiccate greater amounts of seed within an airtight commercially available vessel, such as a sealable plastic bucket or insulated beverage cooler.

Seed viability of torrid panicgrass remained stable over the duration of the experiment when equilibrated to 12% RH; however, as RH increased (50% and 75%), seed viability decreased. Ultimate seed survival is

Fig. 2. Seed viability maintenance of torrid panicgrass seeds exposed to varying levels of relative humidity (RH) and temperature over a 10-month storage duration. Data are represented over two seed batches and plotted with SE bars; $1.8 \times ^\circ\text{C} + 32 = ^\circ\text{F}$.

directly related to the time the seed has been exposed to unfavorable conditions of temperature or humidity (Barton, 1961; Harrington, 1972; Roberts, 1972). Storage parameters can greatly impact seeds both in terms of relief of dormancy and persistence over time (Priestley, 1986).

In summary, exogenous chemical treatments did not relieve torrid panicgrass dormancy; however, seed germination was elevated from 0% to 4% to a maximum of 55% germination by storage maintained at 12% RH at 30 °C for a duration of 8 months. Torrid panicgrass seeds can maintain viability for up to 10 months when a storage RH of 12% is imposed and consistently maintained.

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