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Testa imposed dormancy in *Vallisneria americana* seeds from the Mississippi Gulf Coast[1]

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KAUTH, P. J. AND P. D. BIBER. (Center for Plant Restoration and Coastal Plant Research, Gulf Coast Research Laboratory, The University of Southern Mississippi, Ocean Springs, MS 39564). Testa imposed dormancy in *Vallisneria americana* seeds from the Mississippi Gulf Coast. J. Torrey Bot. Soc. 141: 80–90. 2014.—In response to hurricane and oil-spill environmental impacts along the northern Gulf of Mexico, coastal and marine habitat restoration has become a priority. In particular, restoration of submerged aquatic plants is vital for ecosystem health. To facilitate restoration, developing propagation protocols for Gulf coast plants is necessary, but challenging due to the lack of information on many species. Previous seed germination research of *Vallisneria americana*, a submerged aquatic species with declining abundance in coastal habitats, from northern latitudinal populations reported germination percentages between 80–90%. Germination experiments using Mississippi Gulf coast plants revealed unexpected outcomes. Less than 8% germination occurred when seeds were germinated in a 16 hr photoperiod or 24 hr dark period at 10, 20, 30 or 40 °C. To enhance germination, cold stratification and gibberellic acid soak treatments were conducted, but germination was below 10%. A subsequent seed scarification experiment was conducted that resulted in 90% germination when incubated at 30 °C. In addition, an imbibition experiment revealed that both scarified and non-scarified seeds imbibed water. Due to this imbibition, *V. americana* seeds used in this study were considered to be physiologically dormant. Refining existing seed-based propagation protocols is recommended to ensure the success of revegetation in restoration projects.

Key words: ecotype, germination ecology, physiological dormancy, submerged aquatic vegetation.

Vallisneria americana Michx. is a submerged aquatic macrophyte (i.e., submerged aquatic vegetation or SAV) found in fresh and brackish waters. The plant serves as a food and habitat source for waterfowl, fish, and invertebrates, stabilizes sediment, oxygenates water, and aids in improving water quality (Rogers et al. 1995, Wigand et al. 2000, Smart et al. 2005, McFarland 2006). The distribution of *V. americana* in eastern North America is from southern Canada to the Gulf of Mexico and from the Atlantic coast west to Texas, Oklahoma, and South/North Dakota (Haynes 2000, McFarland 2006). In western North America, it is found in British Columbia, Canada (Catling et al. 1994) as well as Washington, Idaho, Oregon, Nevada, Arizona, and New Mexico (McFarland 2006). *Vallisneria americana* is perennial and dioecious, and relies heavily on clonal reproduction via stolons during the growing season, and overwintering tubers and buds during the dormant season (Lokker et al. 1997). However, sexual reproduction and seed production are also important in this species and serve to maintain genetic diversity (Kimber et al. 1995, McFarland 2006), function as dispersal units (Lokker et al. 1997), and provide propagules for recolonization (Kimber et al. 1995, Lokker et al. 1997).

Previous research on seed germination of *V. americana* indicated no pretreatments were required to achieve near 100% germination between 20–30 °C (Jarvis and Moore 2008), and no seed dormancy issues have been reported (Lokker et al. 1997) in these northern populations. While Kimber et al. (1995) found that dark conditions promoted increased germination over light conditions, no differences between light and dark conditions have been reported as well (Jarvis and Moore 2008). Ferasol et al. (1995) found that mature cold-stratified as well as scarified seeds germinated to slightly higher percentages than either non cold-treated or non-scarified seeds, however, cold-stratification and scarification treatments

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were not essential for germination to occur. Ferasol et al. (1995) concluded that the seed testa acted as a barrier to water, oxygen, or carbon dioxide, however, they provided little information on water imbibition of mature, scarified, or non-scarified seeds.

Although information exists on *V. americana* seed germination in northern populations, no information is available on germination of Gulf coast populations at the southern end of the species' distribution. Previous research investigated germination of populations from southwest Ontario (Ferasol et al. 1995, Lokker et al. 1997), western Wisconsin (Kimber et al. 1995), Chesapeake Bay (Jarvis and Moore 2008), and New York (Muenscher 1936). Studying seed germination ecology of *V. americana* could be useful to determine the extent of ecotypic differentiation across the range of this widely distributed SAV species.

Understanding the germination ecology of individual or regional populations is essential to determine environmental cues that break seed dormancy and promote germination (Baskin and Baskin 2001, Donohue 2005), given that different populations of the same species often have contrasting requirements for germination. For example, Seneca (1972) identified three eco-regions for *Uniola paniculata* L. through germination studies including Virginia and North Carolina populations, Florida Atlantic populations, and Gulf coast populations. Seneca (1974) also identified four eco-regions for *Spartina alterniflora* Loisel. including New England, Mid Atlantic, South Atlantic, and the Gulf of Mexico coast. While Seneca (1974) did not report major differences in germination due to temperature within populations, he noted that caryopses from Mississippi germinated best at cooler day and night temperatures. Probert et al. (1985) found that northern European *Dactylis glomerata* L. ecotypes required both light and alternating temperatures for germination, while more southerly Mediterranean ecotypes germinated in continual darkness and constant temperatures. In addition to germination, seed dormancy patterns among ecotypes of the same species often differ as well. *Penstemon eatonii* A. Gray ecotypes from sites with colder winters required longer chill periods before germination began, and were slower to germinate than seeds from sites with warmer winters (Meyer 1992). Smart and Dorman (1993) suggested that northern (Wisconsin) and southern (Texas) *V. americana* ecotypes exist with differing growth strategies, but further information about distribution and seed germination ecology was not reported.

In a preliminary study using standard temperatures from Jarvis and Moore (2008), possible dormancy issues in the local Mississippi (MS) *V. americana* populations were noted. Research on other SAVs has previously suggested the presence of testa-imposed dormancy or barriers to germination (Van Viersen et al. 1984, Koch and Seeliger 1988, Kantrud 1991, Orth et al. 2000, Figuerola et al. 2002), but whether these species exhibit either physical or physiological dormancy remains uncertain. A series of experiments was performed to determine whether *V. americana* seeds from the MS Gulf coast showed signs of physical or physiological dormancy. Physically dormant seeds can not imbibe water, while physiologically dormant seeds can imbibe water (some only to 20–30% fresh mass) but embryo growth can be restricted by the testa (Baskin et al. 2006). Our hypothesis was that *V. americana* seeds from the MS Gulf coast are dormant, and require a dormancy breaking treatment in order to maximize germination.

Materials and Methods. Seed Collection and Experimental Preparation. Mature seed capsules from were collected from plants grown in circular tanks (3.66 m diameter × 0.91 m high) located at the Gulf Coast Research Laboratory, Ocean Springs, MS. Water levels in the tanks were maintained around 0.5 m. The tanks were under shade cloth (50% shading), but exposed to natural temperature and photoperiod conditions. Additional capsules were collected from a naturally occurring population in Fort Bayou (30° 25′ 19.5″ N, 88° 47′ 21.3″ W) near Ocean Springs, MS. Upon collection, seeds were immediately removed from capsules and placed in water at room temperature to allow the mucilage to naturally degrade (5–7 d) before seeds were used for germination experiments. Eight to ten seed capsules were collected before each experiment, and seeds were pooled from all collected capsules. At the time of collection for each experiment capsules were collected from at least 10 individual plants. However, determining the number of plants collected from was difficult due to clonal growth, water depth, and interweaving

of plants. Prior to germination experiments, seeds were agitated alternately twice in 95% ethanol and distilled water for 3 min to reduce potential seed pathogens (Seeliger et al. 1984).

Before starting the germination tests, embryo viability was assessed using a 1% 2,3,5-triphenyl tetrazolium chloride (TTC) solution (Lakon 1949). Testae were nicked at the distal end of the seed to facilitate embryo staining. Seeds were then incubated at 30 °C in the dark for 48 hr before examination. Three replications of 75 seeds each were used for tank-grown plants and three replications of 25 seeds each were used for the Fort Bayou plants. Embryos were considered viable if any degree of red staining was observed.

Three replications of 50 seeds were used per treatment for all germination experiments. Seeds were placed in 100 × 15 mm Petri dishes with 20–25 ml water, and incubated in VWR Signature™ Diurnal Growth Chambers (model 2015, VWR International, Radnor, PA, USA). Water was replenished as needed. A 16 hr photoperiod was provided by GE Ecolux 4100k cool-white fluorescent tubes (F32T8SP41, General Electric, Fairfield, Connecticut, USA).

EXPERIMENT 1: EFFECTS OF TEMPERATURE AND LIGHT ON GERMINATION. Petri dishes were incubated at 13.6 ± 4.9, 22.0 ± 2.1, 30.1 ± 2.2, and 39 ± 4.0 °C for 6 wks. Although nonfreezing temperatures were utilized, the growth chamber at 13 °C formed ice near the compressor. To prevent ice formation in the low temperature chamber, a one hour increase in temperature to 30 °C occurred during the dark photoperiod. Three Petri dish replications per temperature treatment were wrapped in aluminum foil to prevent light exposure, and three were exposed to lighted conditions. To avoid any effect of light on the dark incubated seeds, they were inspected only at the end of the experiment. Average light levels in the growth chambers were as follows: 112.2 ± 24.4 μmol m^{-2} s^{-1} (10 °C), 120.3 ± 22.2 μmol m^{-2} s^{-1} (20 °C), 127.0 ± 27.2 μmol m^{-2} s^{-1} (30 °C), and 113.8 ± 22.4 μmol m^{-2} s^{-1} (40 °C). Germination was monitored every 2–3 d for 6 wks.

EXPERIMENT 2: EFFECTS OF COLD STRATIFICATION ON GERMINATION. A cold-stratification experiment was performed to test whether mature *V. americana* seeds were physiologically dormant. After seed inoculation, Petri dishes were wrapped in aluminum foil and placed in complete darkness at 3.0 ± 1.6 °C for 2, 4, 6, and 8 wks. A control of no chill (30 °C) was also used. After each designated cold-stratification period, vessels were removed, unwrapped, and maintained in a 16 h photoperiod under cool-white fluorescent lights (127.0 ± 27.2 μmol m^{-2} s^{-1}) at 30.1 ± 2.2 °C. Culture vessels were initially scored for signs of germination upon removal from the cold-stratification period. Germination was monitored every 2–3 d for 4 wks after culture vessels were removed from cold storage.

EXPERIMENT 3: EFFECTS OF GIBBERELLIC ACID ON GERMINATION. Simultaneous to the cold-stratification experiment, the presence of physiological dormancy in mature *V. americana* seeds was also tested using a gibberellic acid (GA) soak. Mature seeds were soaked in 100 ml aqueous GA_3 solutions at 0, 10, 25, 50 and 100 μM. Seeds were placed in 250 ml Erlenmeyer flasks, wrapped in foil to exclude light, and incubated at 10 °C for 48 h. Seeds were then removed from the solutions, placed in Petri dishes, and incubated in a 16 hr photoperiod under cool-white fluorescent lights (127.0 ± 27.2 μmol m^{-2} s^{-1}) at 30.1 ± 2.2 °C. Germination was monitored every 2–3 d for 4 wks.

EXPERIMENT 4: EFFECTS OF SCARIFICATION ON GERMINATION AND WATER UPTAKE. To test for the presence of physical dormancy, the distal end of mature seeds was nicked with a scalpel blade prior to germination. Scarified and non-scarified seeds were then incubated at the temperature and light conditions mentioned in experiment 1. Germination was monitored every 2–3 d for 4 wks.

A water imbibition experiment was also conducted to determine if intact, mature seeds could imbibe water. Seeds were either punctured at the distal end with a needle (scarified) or left intact (non-scarified). The dry mass of three replications of 50 seeds was measured prior to imbibition. Seeds were then placed into Petri dishes with moistened filter paper. After 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, and 48 h seeds were removed, lightly blotted, weighed to determine fresh mass, and returned to their respective Petri dishes.

DATA COLLECTION AND ANALYSIS. At the end of each experiment, final germination percentages were calculated by dividing the number of

Table 1. Results of the Shapiro-Wilk test for data normality for *Vallisneria americana* seed germination in four experiments. Germination and mean germination time data were tested from each experiment. Non-normally distributed data were subsequently arcsine transformed and retested for normality. Data with significance below $\alpha = 0.05$ are non-normally distributed.

	Germination			Mean germination time		
Experiment	Test statistic	d.f.	*P*	Test statistic	d.f.	*P*
1	0.92	12	0.32	0.91	12	0.22
2	0.91	18	0.07	0.91	18	0.10
3	0.73	15	**0.001**	0.75	15	**0.01**
4	0.83	24	**0.001**	0.83	24	**0.001**

germinated seeds by the total number of seeds per replication. Mean germination time was also calculated according to Ellis and Roberts (1980):

$$\mathrm{MGT} = \sum (nT)/N$$

where n is the number of seeds newly germinated at time T; T is the time (days) from beginning of germination test; and N is the final number of germinated seeds.

Prior to analysis, data were tested for normality using Shapiro-Wilk's test for normality (Shapiro and Wilk 1965) in SPSS v. 20 (IBM Corp. 2011). Germination data were arcsine transformed in Microsoft EXCEL prior to analysis, but non-transformed means are presented. Experiment 1 was analyzed using a three-way ANOVA to account for the temperature, light, and seed source interactions followed by *post hoc* LSmeans test. Experiment 2 was analyzed using a one-way ANOVA followed by a *post hoc* LSD test. Experiment 3, due to non-normality, was analyzed with a Kruskal-Wallis non-parametric analysis of variance test (Kruskal and Wallis 1952) followed by a *post hoc* pairwise comparison test. Experiment 4 data, which were also non-normally distributed, were analyzed using the Scheirer-Ray-Hare extension (Scheirer et al. 1976) of the Kruskal-Wallis test, which is a two-way ANOVA on ranked data. The imbibition portion of experiment 4 was analyzed using a repeated measures ANOVA test and a *post hoc* LSmeans test to assess differences at the various time points. Analysis of data for experiments 1, 2, and 4 was performed using SAS v. 9.1 software (SAS Institute Inc. 2008), while experiment 3 was analyzed using SPSS. The significance level for all experiments was $\alpha = 0.05$.

Results. Mean embryo viability measured by TTC staining was 74.7 ± 4.7% for the wild collected Fort Bayou population and 98.3 ± 0.9% for tank collected seeds. In addition, differences were observed between TTC embryo viability and germination (86–91% depending on treatment) of tank-collected seeds. Shapiro-Wilk's test revealed normally distributed data from experiments 1 and 2, but non-normally distributed data in experiments 3 and 4, even after transformation (Table 1).

For experiment 1, only temperature had a significant effect on germination, while temperature and the interaction between temperature and seed source influenced MGT (Table 2). Germination percentages were low, with 7.3% being the maximum germination in several treatments (Fig. 1A). Germination of the tank-collected seeds was slower at 40 °C while germination of wild-collected seeds was faster at 30 °C compared to 10 or 40 °C (Fig. 1B). In this temperature range, the majority of seeds germinated between 8–12 d upon incubation.

Surprisingly, cold-stratification did not increase germination (Table 2). In fact, the control at room temperature had the highest germination (9.3%), but it was not statistically greater than the five stratification treatments (Fig. 2). MGT also was not significantly affected by the duration of cold-stratification, although longer chilling periods tended to decrease the number of days to reach mean germination (Fig. 2). Likewise, GA did not increase germination compared to the control or to the percentages reported from experiment 1. Although germination was significantly different among the GA treatments (Table 2), maximum germination was only 6.7% in the 50 μM treatment (Fig. 3). MGT was statistically similar among the GA treatments (Fig. 3).

Scarification substantially improved germination success, and temperature and the interaction between scarification and temperature

Table 2. Test results for *Vallisneria americana* seed germination in four experiments, reporting germination percentage (Germination) and mean germination time (MGT). Experiments 1 and 2 represent ANOVA results, while 3 and 4 represent Kruskal-Wallis and Scheirer-Ray-Hare test results, respectively.

			Germination		MGT	
Experiment	Source	d.f.	*F*	*P*	*F*	*P*
1	Temperature	3	3.43	**0.03**	10.0	**0.0006**
	Light	1	0.52	0.48		
	Temp*light	3	0.69	0.56		
	Seed source	1	2.21	0.15	1.94	0.18
	Temp*seed source	3	0.77	0.52	3.68	**0.03**
	Light*seed source	1	0.76	0.39		
	Temp*light*source	3	0.07	0.98		
2	Cold period	5	0.52	0.75	1.33	0.32
3	GA concentration	4	10.4	**0.04**	6.47	0.17
4a (germ test)	Temperature	3	15.8	**<0.0001**	8.49	**0.001**
	Scarified	1	224.1	**<0.0001**	12.1	**0.003**
	Temp*Scarif	3	9.02	**0.001**	8.43	**0.001**
4b (imbibition)	Scarification	1	57.9	**0.002**		
	Time	12	61.9	**<0.0001**		
	Scarif*Time	12	18.1	**<0.0001**		

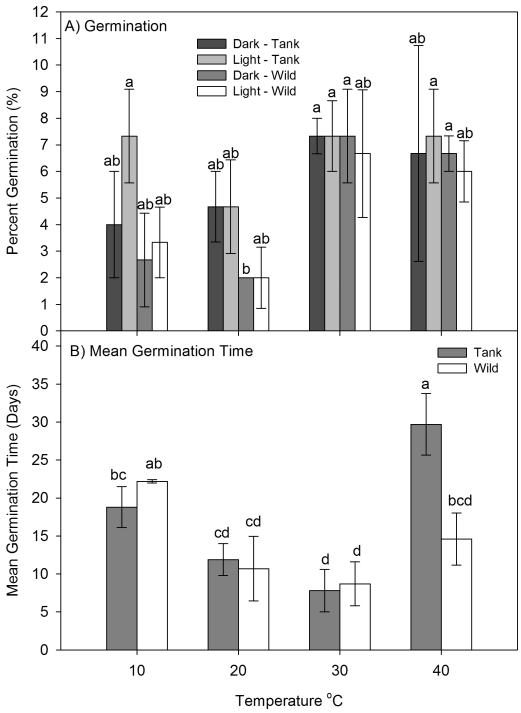

FIG. 1. Seed germination of *Vallisneria americana* influenced by light, temperature, and seed source (wild vs. tank collected seeds) after 6 weeks. A) Final percent germination at 10, 20, 30 or 40 °C in either a 16 hr photoperiod or continual darkness. B) Mean germination time as a measure of germination rate. Histobars represent the mean of 3 replications with 50 seeds ± SE. Histobars with the same letters are not significantly different at $\alpha = 0.05$.

significantly influenced germination (Table 2). Germination of scarified seeds, which was between 86–91%, was higher at 20–30 °C compared to 10 °C and 40 °C (Fig. 4A). Still, the lowest germination of scarified seeds was approximately 10 times that of non-scarified seeds. MGT of scarified seeds was much lower at 10 °C and 40 °C compared to non-scarified seeds (Fig. 4B). However, at 20 °C and 30 °C no differences were observed between scarified and non-scarified seeds (Fig. 4B).

While fresh mass increased for both scarified and non-scarified seeds (Fig. 5), fresh mass increase of scarified seeds was significantly greater than non-scarified seeds at all times (Table 2). Scarified seeds quickly imbibed water, and their mass was greater than non-scarified seeds by 0.25 hr and almost three-fold greater at 48 hr. The imbibition observed in the non-scarified seeds indicates that *V. americana* seeds are not physically dormant, but do have some level of dormancy.

Discussion. While seed germination of *V. americana* has previously been studied, the potential role of physical versus physiological dormancy has not been fully addressed. The potential for testa imposed dormancy in aquatic plants was noted by Crocker (1907), and in their review of the literature Orth et al. (2000) suggested that both physical and physiological dormancy exists in seagrass and SAV seeds. Physical dormancy has only been found in only 15 plant families, two of which

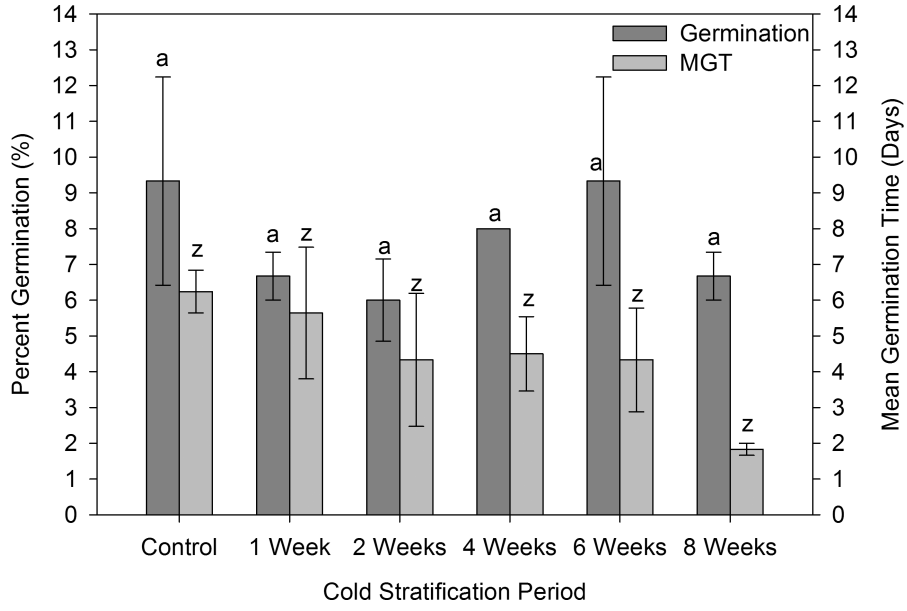

FIG. 2. Effect of cold-stratification on seed germination of *Vallisneria americana* after 4 weeks post-stratification. Histobars represent the mean of 3 replications with 50 seeds ± SE. Histobars with the same letter within a given experimental test are not significantly different at $\alpha = 0.05$.

are monocots and does not include the Hydrocharitaceae to which *V. americana* belongs (Baskin et al. 2000). In this study *V. americana* seeds from the MS Gulf coast were dormant, imbibed water, and scarification enhanced germination success indicating that seeds were physiologically and not physically dormant. Physically dormant seeds are unable

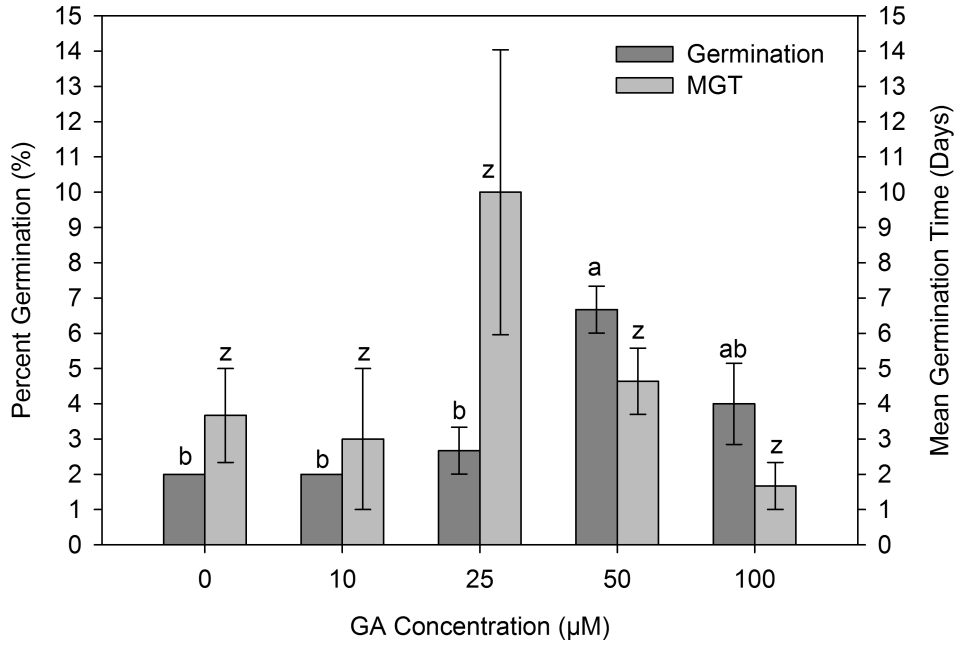

FIG. 3. Effect of gibberellic acid (GA_3) on seed germination of *Vallisneria americana* after 4 weeks post-treatment. Histobars represent the mean of 3 replications with 50 seeds ± SE. Histobars with the same letter within a given experimental test are not significantly different at $\alpha = 0.05$.

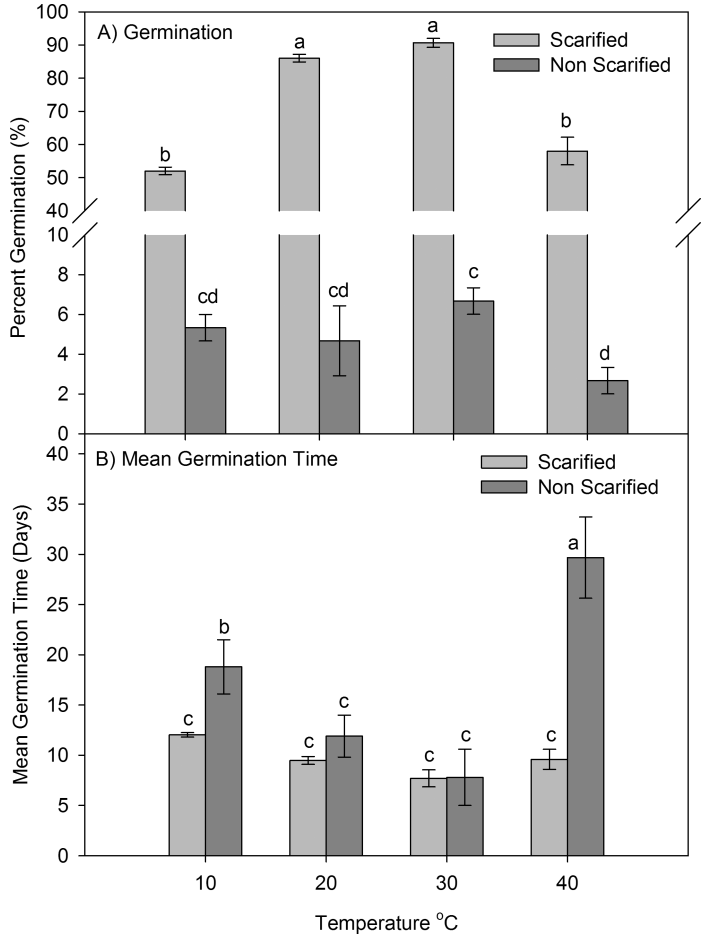

FIG. 4. Effect of scarification on seed germination of *Vallisneria americana* after 4 weeks. A) Final germination percentage. B) Mean germination time as a measure of germination rate. Histobars represent the mean of 3 replications with 50 seeds ± SE. Histobars with the same letter are not significantly different at $\alpha = 0.05$.

to imbibe water without scarification or until a water gap opens in the testa due to an impermeable palisade layer in the testa or fruit coat (Baskin et al. 2000). Due to the imbibition of non-scarified *V. americana* seeds, the embryos likely did not have the physiological growth potential to rupture the testa even after imbibition (Baskin et al 2006). However, *V. americana* seeds from the populations used here had a high degree of dormancy. Low germination of non-scarified seeds, but high embryo viability suggests some level of dormancy as well. In tank-collected seeds, the higher viability (98%) compared to germination of scarified seeds (91%) could be attributed to TTC staining. TTC testing can overestimate viability because this test does not detect inactive enzymes that may become active during germination (Lauzer et al. 1994).

We were surprised that the *V. americana* seeds used did not respond to either the cold-stratification or GA_3 treatments, which are often used to break physiological dormancy (Bewley 1997, Baskin et al. 2006). Use of cold-stratification to increase germination of both submerged and aquatic species has been met with variable success. Cold-stratification has both improved (Van Vierssen et al. 1984) and decreased germination of *Ruppia maritima* L. (Koch and Seeliger 1988, Cho and Biber 2010); increased germination of *Lobelia dortmanna* L. (Farmer and Spence 1987) and *Zizania palustris* L. (Aldridge and Probert 1992); but decreased germination in *Zostera*

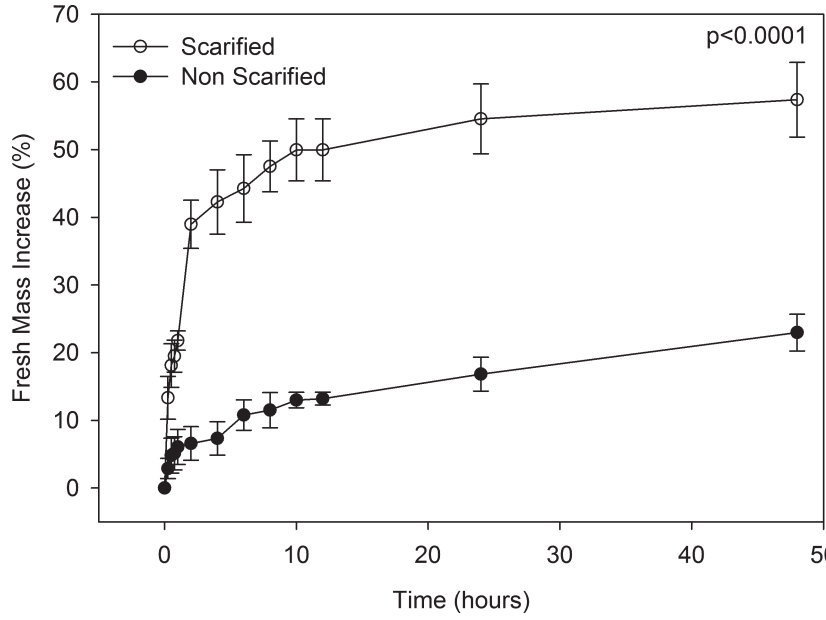

FIG. 5. Effect of scarification on imbibition of *Vallisneria americana* seeds over 48 h. Data points represent the mean of 3 replications each with 50 seeds ± SE. Data between treatments and times were significantly different at $\alpha = 0.05$.

capricorni Ascherson (Conacher et al. 1994), *V. americana* (Ferasol et al. 1995), and *V. australis* S.W.L. Jacobs & Les (Salter et al. 2010). Gibberellic acids have been reported to increase seed germination in aquatic species including *Lobelia dortmanna* (Farmer and Spence 1987), *Zizania palustris* (Cardwell et al. 1978), and *Zostera capricorni* (Conacher et al. 1994). The lack of a chilling response in *V. americana* could be a function of the storage temperature or the storage period, which may have been too short (Ferasol et al. 1995, Salter et al. 2010). The temperature used for cold-stratification was approximately 3 °C, which is colder than the seeds may encounter for prolonged periods in nature in MS. The unresponsive nature of *V. americana* to exogenous GA was likely due to the restrictiveness of the testa, or because endogenous levels of GA were sufficient to trigger germination. In addition, GA would no longer be required to enhance germination once seeds are scarified. Traditional physiological dormancy breaking techniques, such as stratification and GA, will not be successful in this southern ecotype of *V. americana* due to the imposed limitations of the low permeability of the testa.

Scarifying the testa of *V. americana* drastically increased germination by increasing permeability and imbibition. Although Ferasol et al. (1995) found that mechanical scarification more than doubled germination in *V. americana*, it was not essential for germination in their population. However, chemical scarification of *V. spiralis* L. seeds from India was essentially necessary for germination to occur in that species (Choudhuri 1966). Previous research on seed dormancy in other SAVs indicated that both physiological and physical dormancy occurs in *Z. capricorni* (Conacher et al. 1994), *Z. noltii* Hornem. (Loques et al. 1990), and *Z. marina* L. (Harrison 1991) based on findings of increased germination when seeds were scarified. However, physical dormancy likely is not occurring, but rather it is physiological dormancy imposed by the restrictive testa (Probert and Brenchley 1999, Baskin and Baskin 2001). Confusion over physical and physiological dormancy exists, because scarification, used to rupture the impermeable testa, is often considered a treatment to break physical dormancy (Baskin and Baskin 2001). However, true physical dormancy can only be determined through an imbibition experiment (Baskin et al. 2006). Due to the observed imbibition occurring in non-scarified seeds of *V. americana*, physiological dormancy imposed by the testa, rather

than physical dormancy is occurring in *V. americana* seeds from the MS Gulf coast.

The dormancy of *V. americana* seeds from the Gulf coast used in this research was surprising, given that high germination percentages (between 50–90% depending on population) have been reported without pretreatment in other locations (Ferasol et al. 1995, Kimber et al. 1995, Jarvis and Moore 2008). In addition to *V. americana*, the congener *V. natans* (Lour.) Hara germinated to high percentages (> 90%) in light at 25–30 °C (Ke and Li 2006), and germination of three *Vallisneria* species in China was between 25–75% under natural environmental conditions (Zhongqiang et al. 2005). Although the dormancy reported here appears to be novel in this genus, the optimal temperature range between 20–30 °C and the non-significant effect of light on germination was similar to the findings of Jarvis and Moore (2008) from the Chesapeake Bay.

The dormancy issues we report may be characteristic of MS Gulf coast populations or possibly a result of plants maintained in tank culture over multiple years. However, we found the same dormancy issues in the wild collected seeds, and germination was not significantly different between sources. Ecotypes and biotypes have been reported previously for *V. americana* (Smart and Dorman 1993, Smart et al. 2005, Gettys and Haller 2010), therefore, seeds used in our experiments could be of an ecotype whose germination has not yet been reported. An alternative explanation may be that the *Vallisneria* species found in coastal bays and estuaries along the Gulf coast has been misidentified in the past. Conflicting information regarding the differentiation and distribution of *V. americana* and *V. neotropicalis* Marie-Vict. exists. While Les et al. (2008) recognize *V. neotropicalis* as a distinct species based on genetic differences, ITIS (2013) does not formally recognize *V. neotropicalis* as a distinct species. *Vallisneria neotropicalis* has been reported recently from Mobile Bay, AL (Lafabrie et al. 2011a, Lafabrie et al. 2011b), which is approximately 40 km from Ocean Springs, MS, based largely on morphological attributes. It is, therefore, possible that the novel finding of dormancy in the MS Gulf coast population is actually a result for *V. neotropicalis*, which has historically been incorrectly identified as *V. americana*. Further taxonomic research is required to elucidate the differences between these two species.

The presence of dormancy in the Gulf coast population could alternatively be related to the ecological role of the seed bank. While *V. americana* does form a seed bank at varying depths (Kimber et al. 1995, Lokker et al. 1997, Jarvis and Moore 2008), seed and seed capsule longevity *in situ* has not been studied to date. However, seed capsules, which are typically produced from May–November along the MS Gulf coast, can remain intact longer than 18 months when stored under water in laboratory and natural conditions (pers. obs). The long seed capsule maturation period may ensure that testae have weakened sufficiently to allow imbibition, and seeds are ready to germinate. The seed capsule production period occurs during months of frequent disturbances (e.g., hurricanes, tropical storms) in this region. Seeds shed at this time may have a dormancy requirement in order to germinate under more favorable conditions in the following year. Dormancy could also be related to a reproductive strategy in southern ecotypes that do not produce winter buds (Smart and Dorman 1993); dormant seeds may survive dispersal events more efficiently than non-dormant seeds.

Based on our results, seed dormancy is a key characteristic of MS Gulf coast *V. americana* plants, and must be relieved in order to establish efficient propagation protocols. SAV reproduction via seeds is not well researched, yet vital to understand their role in maintaining genetic diversity, dispersal, recruitment, and maintenance and establishment of current and new populations (Orth et al. 2000). For successful restoration projects, developing more efficient and thorough seed-based propagation protocols for locally adapted SAVs is crucial (McFarland 2006, Lloyd et al. 2012). Using seed propagation to produce plants assists in maintaining genetic diversity and reducing inbreeding depression in *V. americana* restoration sites (Lloyd et al. 2012). To ensure the long-term survival of *V. americana*, future research should include studies on seed longevity, seed bank analysis, and refined seed propagation protocols for local ecotypes.

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