Aquatic Botany 120 (2014) 297–303

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Moisture content, temperature, and relative humidity influence seed storage and subsequent survival and germination of *Vallisneria americana* seeds

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ARTICLE INFO

Article history:
Received 26 July 2013
Received in revised form
18 September 2014
Accepted 22 September 2014
Available online 28 September 2014

Keywords:
Aquatic plant
Germination ecology
Moisture content
Submerged aquatic vegetation

ABSTRACT

Loss of aquatic plant species has occurred in coastal and freshwater habitats throughout the world. In particular, population and habit loss of the submerged aquatic species, *Vallisneria americana* Michx., have made it a potential restoration candidate. For successful restoration, reliable seed sources are vital for producing plants or for direct seeding. Seed survival and their ability to retain viability through storage are key components for successful reproduction. Seed storage and subsequent germination of *V. americana* were studied over a 6-month period to determine the conditions that retain seed vigor. Seeds were stored at three humidity levels 11%, 50%, 95% using saturated salts and ambient humidity for 1, 2, 4, and 6 months at either cold or ambient temperatures. After storage intervals, seed moisture content, embryo viability, and germination were determined. Timing of germination and seed survival were analyzed using semi- and non-parametric analyses. Storing seeds at lower RH levels for longer periods reduced seed survival and resulted in later germination. Germination and seed survival increased in seeds stored at higher relative humidity and led to earlier germination. Seeds of *V. americana* from the northern Gulf of Mexico were shown to be desiccation tolerant and have orthodox seed characteristics.

1. Introduction

Vallisneria americana Michx. is a perennial, submerged aquatic macrophyte, found in coastal and freshwater aquatic ecosystems throughout North America, and is a focal species for restoration in the Chesapeake Bay and Gulf of Mexico regions (Lloyd et al., 2012). Due to natural and anthropogenic events (Short and Wyllie-Echeverria, 1996; Short and Neckles, 1999; Hay et al., 2000), maintaining germplasm (e.g. seeds) is necessary when natural seed production is low or unavailable (Ailstock and Shafer, 2006). In particular, determining proper seed storage techniques is vital to retain viability through desiccation and subsequent storage (Young and Young, 1986; Tweddle et al., 2003).

Seed storage can be challenging given the inherent nature of seeds. Orthodox seeds can survive desiccation to 3–7% moisture content and temperatures as low as −20 °C for an indefinite period (Hay et al., 2000). Recalcitrant or unorthodox seeds cannot germinate when desiccated below 20–40% moisture content (Hay et al., 2000). Still other seeds have intermediate storage characteristics and can be dried to 12–14% moisture content, but cannot be stored cold upon desiccation (Hay et al., 2000; Bonner, 2008). While unorthodox storage characteristics in aquatic seeds is common, orthodoxy may be more frequent than previously thought (Hay et al., 2000).

Seed storage of *V. americana* has been previously studied, but debate on the subject has not been concluded. Muenscher (1936) found that mature seeds could not be desiccated in storage without damaging the embryo, but seeds remained viable when intact capsules were stored at 4 °C for up to 3 years (Ferasol et al., 1995; Campbell, 2005; Moore and Jarvis, 2007). In a related species, *V. australis* S.W.L Jacobs & D.H. Les, from central Victoria, Australia, drying seeds up to 8 months more than doubled the final germination percentage but also delayed germination compared to wet-stored seeds (Salter et al., 2010).

Here we describe a technique to desiccate and store *V. americana* seeds under different relative humidity (RH) levels and temperatures. Although seed storage of *V. americana* is unclassified, this species may have non-orthodox seed storage characteristics (Hay et al., 2000). Seeds can remain viable when capsules are stored

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http://dx.doi.org/10.1016/j.aquabot.2014.09.009
0304-3770/

in water, but doing so can lead to contamination and precocious germination, and timing collection of mature capsules can be challenging. Our objectives were to determine: (1) whether *V. americana* seeds can survive desiccation, (2) conditions that allow *V. americana* seeds to be stored for up to 6 months, and (3) seed storage physiology of *V. americana* seeds.

2. Materials and methods

2.1. Seed source and collection

During November 2012, approximately 75 mature seed capsules were collected from tank-grown plants located at the Gulf Coast Research Laboratory, Ocean Springs, MS. The tanks were under 50% shade cloth, but exposed to natural temperature and photoperiod conditions. Upon collection seeds were immediately removed from capsules, and placed in water at ambient temperature to allow the mucilage to naturally degrade (5–7 days). Prior to experimentation, all seeds were pooled from the collected capsules.

2.2. Experimental design

The seed storage system used was similar to Carpenter et al. (1995). Seeds were placed on a 70 mm diameter filter paper supported by a 60 mm × 15 mm piece of PVC pipe in 100 mm × 25 mm Petri dishes (D943; *Phyto*Technology Laboratories®, LLC, Shawnee Mission, KS). Two Petri dishes (one for germination and viability testing and one for moisture content determination) containing 250 seeds each were prepared per storage treatment (Fig. 1). To reduce storage variability, seeds allocated for germination were pretreated in one dish as per standard methods (Ellis et al., 1982, 1989). Storage treatments were a combination of two temperatures (3.0 °C ± 1.5 or ambient temperature (23.2 °C ± 3.0)), four RH conditions, and four storage storage times (1, 2, 4, or 6 months). The four RH conditions were achieved by allocating 50 ml of a saturated salt solution (95% RH-KNO_3, 50% RH-$Mg(NO_3)_2$, 11% RH-LiCl, ambient of 50.2% ± 9.6 RH) to each dish (Wexler and Hasegawa, 1954; Greenspan, 1977). Petri dishes were sealed with Parafilm® (Pechiney Plastics Packaging Company, Chicago, IL) and stored in continuous darkness.

After designated storage times, seeds from the first Petri dish were dried at 130 °C for 1 h and cooled for 45 min at 31.3% ± 7.4 RH (International Seed Testing Association, 1985). Seed moisture content of two replications (125 seeds each) was determined using the following equation:

$$(M2 - M3) \times \frac{100}{M2 - M1}$$

where M1 is the weight in grams of the container and its cover; M2 is the weight in grams of the container, its cover, and seeds before drying; and M3 is the weight in grams of the container, its cover, and seeds after drying.

From the second Petri dish, seeds were randomly selected for viability and germination testing. Embryo viability of 100 seeds was tested after designated storage times using a 1% 2,3,5-triphenyl tetrazolium chloride solution (Lakon, 1949). To facilitate embryo staining, seed coats were nicked at the distal end of the seed with a razor blade. Seeds were incubated at 30 °C in the dark for 48 h before examination. Embryos were considered viable if any degree of red staining was observed.

Germination tests were conducted by monitoring three replications of 50 seeds for each storage treatment combination (temperature × RH × storage time). Seeds were agitated alternately twice in 95% ethanol and distilled water for 3 min to reduce potential contamination (Seeliger et al., 1984). Seeds were then placed in 100 mm × 15 mm petri dishes with 20–25 ml water, and incubated in VWR Signature™ Diurnal Growth Chambers (model 2015, VWR International, Radnor, PA, USA) with a 16 h photoperiod (GE Ecolux 4100k cool-white fluorescent tubes; F32T8SP41; General Electric, Fairfield, CT, USA) at 30.1 °C ± 2.2. Water was replenished as needed.

2.3. Data collection and analysis

Temperature and RH levels were monitored throughout the experiment using HOBO HO8-003-02 data loggers (Onset Computer Corporation, Bourne, MA). Germination was monitored every 2–3 days for 30 days. Due to a large percentage of censured data (no germination), semi-parametric and non-parametric time-to-event analyses were conducted (McNair et al., 2012; Perez and Kettner, 2013). After the experimental period, germinated seeds were assigned an event code of '1' and non-germinated seeds were assigned '0'.

The non-parametric Kaplan–Meier estimator of survivor function (Kaplan and Meier, 1958) was used to determine how likely seeds survived during the course of the experiment. Main effects (RH, month, temperature) along with temperature/RH interactions within months were tested. Data were log-ranked and the Holm–Sidak post hoc multiple comparison test was used to compare final survival data. Kaplan–Meier statistics were generated in both R v. 2.12 and SigmaPlot v. 12, while Pearson's correlation test was performed in SigmaPlot v. 12.

The semi-parametric Cox proportional hazards model (Cox, 1972) was used to determine how likely it is that an ungerminated seed will germinate within the experimental time frame (30 days). This is referred to as the hazard function or rate and replaces traditional measures such as germination rate or mean germination time. A forward model building approach was used in R v. 2.12 (http://www.r-project.org/) to determine the combination of the factors (including interaction effects) that gave the best overall Cox model using the fewest variables. A three factor model using month, relative humidity, and temperature was determined to be the best fit model based on the Likelihood Ratio. Using the Cox regression proportional hazards model function in SigmaPlot v. 12 (Systat Software, Inc, Chicago, IL), a Chi-square test with a Holm–Sidak correction was used to compare main effects (month, temperature, treatment) to the reference group. Once the hazard ratio coefficients were calculated, the values were used to construct a look up table in Excel 2010 (Microsoft Inc, Redmond, WA, USA)

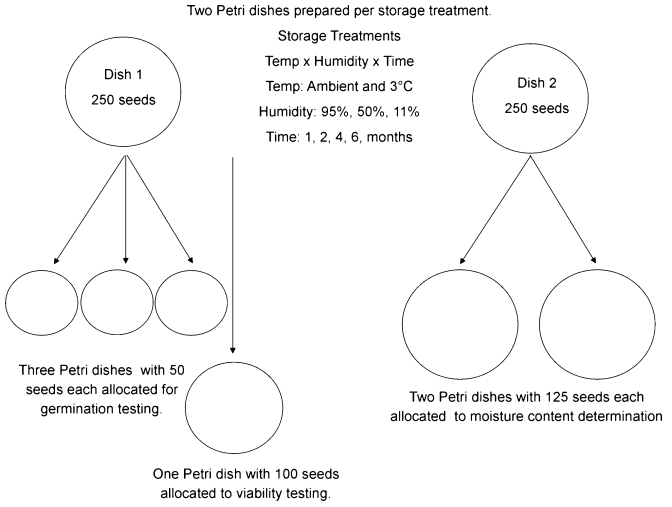

Fig. 1. Outline of experimental procedures.