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Effect of overwinter hydration, seed storage time, temperature, photoperiod, water depth, and scarification on seed germination of some *Schoenoplectus*, *Polygonum*, *Eleocharis* and *Alisma* species

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ABSTRACT

In an effort to improve fish habitat in western irrigation reservoirs, germination of seeds of six wetland plant species was investigated in two separate experiments. In the first, seeds were stored overwinter at 3–4 °C either dry or wet. In spring, these groups were further divided into four constant temperature treatments (15, 20, 25, or 30 °C) and 3 fluctuating temperature treatments (low, moderate, high). Within each treatment, seeds from a subset of species (*Alisma gramineum, Schoenoplectus acutus, S. americanus*) were scarified using either bleach for 1 or 5 d or mechanical abrasion. Seeds were exposed to a 12 h photoperiod in Petri plates within germination chambers for 57 days. In the second experiment, photoperiod (12, 14, or 24 h light), water depth (0 or 4 cm; *S. acutus* only), temperature, and bleach pretreatment were further evaluated. Cold, wet storage (CWS) and bleach treatment significantly improved germination (84 to 100%) of *A. gramineum* and led to quicker germination. Bleach treatment for 5 days significantly increased germination (13%) for *S. acutus* at 15 °C. Temperature effects varied with species. Inundation of seeds significantly improved germination of *S. acutus*. For both *Schoenoplectus* species, germination was highest at daily fluctuating temperatures of 32–38 °C. Photoperiod effects were inconsistent. *Polygonum pensylvanicum* germination was only evaluated in the second experiment; maximum germination (54%) was under a diel cycle of 15–20 °C after 1 year of CWS. Based on the study, effects of temperature, temperature fluctuation, water depth, overwinter storage conditions, and scarification are sufficiently known to use the data for initiating seed pretreatment for habitat projects.

1. Introduction

In western irrigation reservoirs, where large water level fluctuations are common, aquatic macrophytes are largely absent, resulting in a need for more fish and invertebrate habitat. Wetland and aquatic plants could help address the need, as well as provide food and shelter for waterfowl, songbirds, and other animals (O'Neill, 1972). Aquatic plants support higher fish densities, reduce the risk of predation, and provide cover and habitat for species that rely on structure (Savino and Stein, 1982; Dibble et al., 1996). Aquatic macrophytes can also play a role in the control of algal blooms, shifting nutrients from phytoplankton to macrophytes (Hasler and Jones, 1949; Ibelings et al., 2007). By establishing desirable aquatic macrophytes, the spread of undesirable weedy species can be reduced (Smart et al., 1994). Some macrophytes such as *Schoenoplectus americanus* can also remove heavy metals from sediment (Santos-Díaz and Barrón-Cruz, 2011).

Propagation and establishment of aquatic plants for fisheries and wildlife have been ongoing in the southeastern U.S. (Fowler and Maddox, 1974; Smart et al., 1996, 1998; Fleming, 2010; Webb et al., 2012). Efforts in western U.S. reservoirs have largely focused on artificial habitat rather than wetland plants (Uberuaga and Bizios, 1991; Rogers and Bergersen, 1999). One exception was by Ratcliff and Wurtsbaugh (2009) who noted transient improvement in habitat by planting cereal barley *Hordeum vulgare* on exposed banks of California's Lake Shasta. Difficulty in getting wetland and aquatic plants seeds to germinate (Muenscher, 1936) has led to propagation effects focused largely on transferring container stock of rhizomes or transplants.

Ideally, seeds could be prepared so that they were just a day or two from germinating when they are sown (Eskandari, 2013). Recent development of a process called Solid Matrix Priming, in which seeds are partially hydrated and brought closer to the point of breaking dormancy, has been used for commercial seed pro-

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duction to shorten germination times and improve germination percentages (see review by Eskandari, 2013). The only research to date on priming of species in this study is by Hock et al. (2006) with *Polygonum pensylvanicum*. Hock et al. (2006) found that cold wet storage and solid matrix priming resulted in 22% germination compared to 1% in controls.

In our study, *Polygonum pensylvanicum, P. amphibium*, *Schoenoplectus acutus, S. americanus*, *Alisma gramineum*, and *Eleocharis palustris* were investigated. These six species occur naturally in Utah and were chosen for further investigation because they could potentially provide fish and invertebrate habitat, but were not considered invasive (e.g., *Phragmites australis*). Water smartweed *P. amphibium* is an aquatic emergent plant that provides cover for fish and other aquatic organisms, as well as providing seeds that feed waterfowl, small birds and mammals (Justice, 1941; Guard, 1995). Pennsylvania smartweed *Polygonum pensylvanicum* is an annual, 30–120 cm tall, typically found on stream banks, shores, and ditch banks; it provides seeds for wildlife and cover for fish when inundated (Larson, 1993). In Utah, *P. pensylvanicum* can be found on exposed banks of some reservoirs after irrigation drawdown. Hardstem bulrush *Schoenoplectus acutus* and common three-square bulrush *S. americanus* are slender emergent, rhizomatous, aquatic perennials that form dense stands in shallow water of marshes, ponds, ditches and lakes (Larson, 1993). *S. americanus* reaches up to a meter tall and *S. acutus* up to 1 to 2–3 m tall (Larson, 1993). Water plantain *Alisma gramineum* is an aquatic perennial emergent forb that is used by a variety of birds and animals (Guard, 1995). Creeping spike-rush *Eleocharis palustris* is a rhizomatous, perennial, grass-like emergent that prefers saturated conditions; it provides food for waterfowl and helps stabilize shorelines from erosion (Guard, 1995).

Our study was directed at evaluating several variables that influence germination of the six species noted. There are a number of factors that may influence germination including initial viability percentage; seed moisture content; seed storage conditions (wet/dry); overwintering temperature; seed oxygen requirements; seasonal dormancy patterns, including seed age effects; effects of light, shading and photoperiod; water depth; planting depth; incubation temperature and temperature fluctuation; nutrient effects; planting season; and scarification (Grime et al., 1981; Gerritsen and Greening, 1989; Baskin et al., 1989, 1993). Of these, we examined the effects of fixed and fluctuating temperatures, chemical and mechanical scarification, water depth, cold wet storage (CWS, simulating over-wintering), photoperiod, and duration of wetted seed storage in two separate experiments.

The benefits of CWS for aquatic plant species have been reported by other authors (Harris and Marshall, 1960; Leck, 1996). Grime et al. (1981) observed higher germination after CWS of *Eleocharis palustris*, and three *Polygonum* species. Isely (1944) also noted that germination for four *Schoenoplectus* species was only obtained after seeds were stored in water at low temperature. Commercially available seeds are typically not stored wet, so we wanted to compare the germination of seeds stored wet versus dry. Scarification, i.e., the nicking or chemical weakening of the pericarp, with mechanical abrasion or chemical treatment has been helpful in achieving germination in seeds that are difficult to germinate (Grime et al., 1981; Thullen and Eberts, 1995; Hoag et al., 2001). Water depth has been shown to improve germination of some plant species such as *Viola lanceolata* (Moore and Keddy, 1988), but not others (Willis and Mitsch, 1995; Kellogg et al., 2003). The age of the seeds or time in cold storage can also have impacts on germination, with some species germinating with no exposure to over-winter temperatures (van der Valk et al., 1999; Grime et al., 1981) and others requiring multiple exposures to cold temperatures to germinate (Isely, 1944; Leck, 1996). Fluctuating temperatures have been shown to improve germination in some species (Grime et al., 1981; Thompson and Grime, 1983; Thullen and Eberts, 1995). In our study, we were interested in evaluating some of these strategies for germination induction for the six species selected. We hypothesized that temperature, scarification, water depth, CWS, photoperiod and seed age would have significant effects on germination.

2. Methods

2.1. Experiment 1

Seeds of *Polygonum amphibium*, *Schoenoplectus* (syn. *Scirpus*) *acutus, S. americanus*, and *Alisma gramineum* were obtained in September 2013 from two Utah sites: Cache County (latitude, longitude coordinates: 41.913367, −111.990474 digital degrees), and from Johnson Reservoir, Sevier County (38.616473, −111.639074). The seeds were air dried for about 1–2 weeks at room temperature. Additional seeds of *S. americanus* and *Eleocharis palustris* were purchased from Granite Seed, Lehi, Utah, USA.

The experimental treatments evaluated simulated winter storage conditions (wet or dry, both at 4–7 °C), temperature, and scarification method. The temperature treatments included four fixed temperatures (15, 20, 25, and 30 °C), as well as three fluctuating temperature treatments with similar daily lows (15–16 °C), but with three different daily maximum temperatures (19, 23, 27 °C; low, moderate, and high fluctuating treatments, respectively). The three scarification treatments were mechanical abrasion (Hoag et al., 2001; Hock et al., 2006) or chemical treatment with 0.05% sodium hypochlorite for either 1 or 5 days (O'Neill, 1972; Clevering, 1995). There were four replicate plates per treatment and species, containing 25 seeds in each. Seeds of *Polygonum amphibium* were in short supply in the first experiment, so some temperature and bleach treatments were not tested (see Table 1 for details) and some only had 3 replicates in each.

On 10 December 2013, the seeds were split into two groups, one stored dry in the refrigerator in manila coin envelopes and the other stored wet. Wet storage consisted of Petri plates with sphagnum moss (1.2–1.4 g/plate) and seeds in 8 mL well water (pH = 7.6, hardness and alkalinity = 200 mg/L), kept in the same refrigerator. Periodic water addition kept the dish hydrated over time. The moss helped keep fungal growth from establishing in the Petri plates, but some fungal growth was developing by late April in all plates except for *S. acutus.* Seeds were transferred from these plates to plates with sand to start the incubation stage of the experiment. Seeds that were obviously rotten were not transferred or used for viability testing.

Scarification was done just before adding seeds to the plates. The mechanical scarification was done by rubbing the seeds en masse on emory cloth sandpaper. This cloth was affixed to a short section of 4-inch diameter PVC pipe to create a bowl that kept the seeds from scattering during the process. For each group of seeds, 3 min of rubbing was conducted. The seeds were separated from the additional chaff by hand on white butcher paper as they were allocated to germination Petri plates. The bleach scarification treatments were done in Petri plates and kept in the refrigerator. The seeds were rinsed with water 3 times prior to transfer of the seeds to germination plates. Controls seeds for the scarification treatments were disinfected for fungus and bacterial disinfection using the protocol of Boscaiu et al. (2011; 70% ethanol for 5 min, followed by 5 min with 30% bleach containing 0.05% Triton X-100, then rinsing thoroughly with de-ionized water).

Germination tests generally followed the protocol of Boscaiu et al. (2011). A layer of sterile sand (20 mL) was put in new sterile 100 mm Petri plates and de-ionized water was added to hydrate, but not immerse, the seeds. The plates, with 25 hand-counted seeds each, were put in germination chambers on 21 May 2014. The