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EFFECTS OF TEMPERATURE, STRATIFICATION, SCARIFICATION, AND SEED ORIGIN ON THE GERMINATION OF *SCIRPUS ACUTUS* MUHL. SEEDS FOR USE IN CONSTRUCTED WETLANDS

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Abstract: Procedures to maximize seed germination of *Scirpus acutus* (hardstem bulrush) from two geographic locations were studied in the laboratory. Pregermination conditions included scarification and stratification at 4 ± 1°C for 0, 2, 4, 8, or 12 weeks while submerged in water. Following the treatments, seeds were placed in night/day temperature regimes of 10/25°C or 18/22°C under a 14-hour photoperiod (≈200 μmol m^{-2} s^{-1} PPFD). A stratification period of at least 2 weeks significantly enhanced germination. Germination was significantly greater at 10/25°C than at 18/22°C. Maximum germination percentages of 97.5 and 92.5 were achieved for seed lots exposed to a 10/25°C temperature regime following a 12-week cold period. Germination percentages of seeds from the two geographic locations did not differ significantly ($P < 0.05$).

Key Words: Seed germination, *Scirpus acutus*, hardstem bulrush, cold stratification, constructed wetlands, marshes

INTRODUCTION

Constructed wetlands for the treatment of municipal wastewater are gaining popularity as the cost of building and operating conventional sewage treatment facilities escalates each year. Small and mid-sized communities find constructed wetlands an especially attractive option because of decreased costs and because wastewater can be cleaned to standards that allow it to be discharged into a receiving stream or natural wetlands or reused for irrigation, industries, or recreation (Pride et al. 1990). Reusing water has become more common as populations increase and droughts occur, especially in the arid Western United States (Eastern Municipal Water District, personal communication, 1991).

The role of aquatic plants in wastewater treatment by constructed wetlands has been studied in detail (USEPA 1980, Gersberg et al. 1986, Reddy and Smith 1987, Hammer 1989, Reddy et al. 1989, Breen 1990, Busnardo et al. 1992). Benefits that plants provide include transfer of oxygen to the root zone, proper substrate for the associated nitrifying microbes, and uptake of nitrogen (USEPA 1980, Rogers et al. 1991). *Scirpus* plants are commonly used in wetland treatment systems because they tend to out-compete other plants and effectively remove nutrients from the water (Gersberg et al. 1986, 1989, Smith 1989, Rogers et al. 1991). *Scirpus acutus* Muhl. (hardstem bulrush) is one species that has been used extensively in the Western United States because it is dominant in wetland plant communities, locally abundant, absorbs large amounts of nutrients, and tolerates water depths up to 1.2 m (G. Waller, personal communication, 1991) and sediment conductivities up to 16 mS (Kadlec 1982). In addition, the hard stem of *S. acutus* withstands breakage better than do stems of other *Scirpus* species (G. Waller, personal communication, 1991), and the species has high wildlife value (Martin and Uhler 1939).

Scirpus acutus has been successfully established in constructed wetlands by harvesting rhizome clumps from a local donor marsh and staking them into their new environment (J. Thullen, unpublished data, 1991). However, this technique is labor-intensive, time consuming, and expensive. Sypulski (1943) suggested that a more efficient and inexpensive method to establish large areas of *Scirpus* would be to plant the sites with seeds, but he cited no references and provided no data to support his statement. Moreover, seed germination without the proper pretreatment can be poor and un-

reliable (Bitterroot Native Growers, Inc. and BNG Consultants, personal communication, 1994). Little work has been done on the effects of environmental conditions on *S. acutus* seed germination, and the studies that have been done did not achieve high germination percentages (Muenscher 1936, Oborn 1938, Isely 1944, Harris and Marshall 1960, Shipley et al. 1989).

A germination study was performed with the assumption that it may lead to reduced overall planting costs. The objectives were to 1) develop a technique to achieve maximum germination in seeds of *S. acutus* and 2) determine if seeds collected from two geographic locations have different germination requirements.

MATERIALS AND METHODS

Seed Collection

Seeds were collected from *S. acutus* plants growing in the dry San Jacinto River bed on the San Jacinto Wildlife Refuge north of Hemet, California on August 15, 1991 ("Hemet" seeds) and from a 0.4-hectare, flood-control pond on the Denver Federal Center in Denver, Colorado on August 26, 1991 ("Denver" seeds). Seed heads were picked from the plants and stored dry in brown paper bags at room temperature (20–22°C) for 4.5 months and then in a dark incubator at 10 ± 1°C for 6 weeks. The seed shaft and stems were removed from the seeds using a winnowing machine at the USDA National Seed Storage Laboratory, Colorado State University, Fort Collins, Colorado on February 4, 1992. Empty seeds were discarded. Clean seeds were stored dry in capped glass vials in a dark incubator at 10 ± 1°C until used in the experiments. Just prior to experimental treatments, seeds were surface-sterilized by a 3-minute immersion in a 1% sodium hypochlorite solution followed by two deionized water rinses.

Weights were compared for the seed lots. A random sample of 50 seeds from each of the two seed lots was dried to a constant weight at 60°C, and seeds were weighed individually to within 10 μg. Mean weights and standard deviations were calculated, and any visible differences in appearance were noted.

Pregermination Treatments

In the initial set of experiments, cold, wet pretreatments (stratification) and scarification were examined for their effects on the germination of two geographically distinct seed lots. The two seed lots were tested separately for each of these variables as described below.

The experiments had three replications containing 10 seeds each and an additional facsimile containing 50 seeds. Seeds were tested in 250-mL, foam-plugged Erlenmeyer flasks containing about 175 mL of dechlorinated water.

The effect of cold stratification was tested by placing seeds into their respective flasks with water and then into a dark refrigerator at 4 ± 1°C for either 0, 2, or 4 weeks. At the appropriate times, the flasks were moved into one of two light- and temperature-controlled chambers for germination tests at either the 10/25°C or 18/22°C night/day temperature regime. Later, the effects of 4, 8, and 12 weeks of cold stratification were examined using 4 replicates of 10 seeds each. Data from the two experimental sets have been combined in the results. The first 4-week treatment is designated by "4a," the second 4-week treatment is designated by "4b."

The effect of scarification (puncturing or removing part of the seed coverings) was tested by placing a single layer of seeds on top of a piece of 150 garnet paper and making 20 passes in one direction with another piece of garnet paper wrapped around a wooden block (instructions from Pat Klein, USDA National Seed Storage Laboratory, February, 1992). The seeds were placed into their respective flasks with water and immediately placed in either of the two temperature regimes with no cold treatment period.

Some seeds without a prior cold treatment (scarified and non-scarified) that had not germinated after 47 days in either of the two temperature regimes were then subjected to a delayed cold treatment. Two flasks from each seed lot and germination temperature received this delayed cold treatment (4 ± 1°C) for 2 weeks before being returned to the chambers. Their subsequent germination percentages were recorded for 3 weeks.

Seed Germination

Seeds were incubated in the light- and temperature-controlled chambers with a 14-hour daily photoperiod (about 200 $\mu mol\ m^{-2}\ s^{-1}$, 400–700 nm, cool white fluorescent and incandescent light). One chamber was set to maintain a temperature of 10°C for 10 hours at night and 25°C during the 14-hour day (10/25°C). The other chamber was set for a temperature of 18°C during the 10-hour night and 22°C during the 14-hour day (18/22°C). All replications in the controlled chambers were evaluated weekly. Germination of seeds and growth of fungi and algae were noted.

Statistical analyses were performed on the germination data using the multi-response permutation procedure (MRPP) described by Mielke et al. (1981a, 1981b, 1982). The MRPP, as applied in this study, incorporated into the P-values differences in the pattern of germination in time as well as differences in germination totals. MRPP is a sensitive and powerful

Table 1. Statistical differences between the *Scirpus acutus* seed germination tests.

	Denver	10/25°C	Scarified	Control 0 Week	Stratification Periods at 4 ± 1°C: 2 Weeks	4a Weeks	4b Weeks	8 Weeks	12 Weeks
Hemet	NS (n = 56)								
18/22°C		*** (n = 40)							
Scarified			—	NS	***	***	***	***	***
0 week—control				—	***	***	***	***	***
2 weeks at 4 ± 1°C					—	**	**	***	***
4a weeks at 4 ± 1°C						—	NS	**	**
4b weeks at 4 ± 1°C							—	NS	*
8 weeks at 4 ± 1°C								—	NS
12 weeks at 4 ± 1°C									—
All replications—NS from each other									

All statistics were performed using the multi-response permutation procedure (MRPP) where n = 16 unless otherwise noted. NS = not significantly different.
* = significantly different, $P < 0.05$.
** = significantly different, $P < 0.01$.
*** = significantly different, $P < 0.001$.

test that allows valid comparisons with fewer samples (J. Medina, personal communication, 1993). Seed weight analysis was performed using ANOVA procedures in SAS software (SAS Institute, Inc. 1985).

RESULTS

Denver seeds weighed 0.8 ± 0.2 mg and Hemet seeds weighed 1.1 ± 0.2 mg ($P < 0.05$). No discernible differences existed in shape or color between the seed lots.

No significant differences existed among replications within treatments ($P < 0.05$), or among the first 4-week cold period set (4a) and the second 4-week set (4b) (Table 1). Consequently, data were pooled for some comparisons.

Seeds incubated without an initial cold treatment, including those that were scarified, had very low germination percentages, and they were not significantly different from each other ($P < 0.05$). At 10/25°C, control and scarified Hemet seeds germinated at 13.8% and 11%, respectively. At 18/22°C, they germinated at 6% and 0%. Ninety-three percent of these seeds had not germinated within 47 days. The Denver seeds failed to germinate under any of these conditions. A delayed 2-week cold treatment induced no additional germination in the Denver subsample, but some additional germination (30% in the 10/25°C and 11% in the 18/22°C temperature regime) was induced in Hemet seeds. Only two replications from each test were used to examine the delayed cold treatment, so these data were not statistically analyzed.

Significantly less germination occurred in the control and scarified groups than in cold-stratified groups ($P \leq 0.001$) (Table 1). As the length of the cold-stratification period increased, so did the percentage of germination (Figure 1). The greater the difference in the amount of time spent in stratification conditions, the greater the germination difference between groups. The only final germination percentages that were not significantly different from the other stratifications (besides the first and second 4-week treatments) were 4b versus the 8-week treatment and the 8-week versus the 12-week treatment. This trend occurred in the Denver and Hemet seeds and in each of the two temperature regimes.

Final germination percentages were significantly higher in the Denver and Hemet seeds at 10/25°C than at the 18/22°C temperature regime. This response occurred under all pregermination conditions (Figure 2). No significant differences existed between the pooled germination data of the Denver seeds versus the Hemet seeds. The highest germination percentage in both seed lots was for seeds exposed to 4°C for 12 weeks and then transferred to the 10/25°C temperature regime, 97.5% in Denver seeds and 92.5% in Hemet seeds.

DISCUSSION

Seeds of *S. acutus* in the present study showed definite germination responses to various conditions. The low germination percentages of seeds in the control group are evidence that the seeds are dormant when harvested in the fall (Mayer and Poljakoff-Mayber 1989). Dormant seeds of many species can be induced to germinate by various kinds of scarification treatments (Hart and Berrie 1966, Roberts and Smith 1977). However, scarification did not increase germination

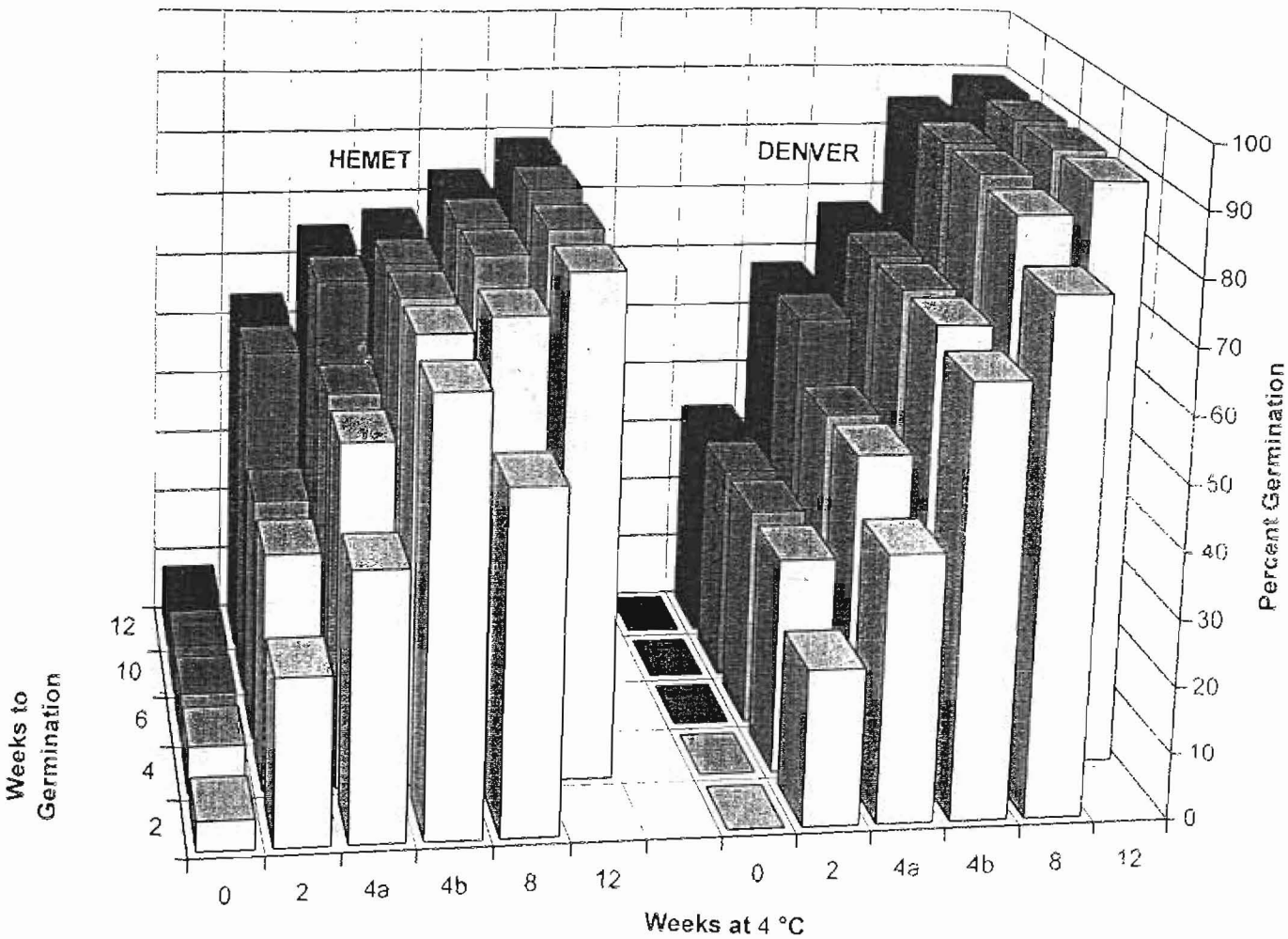

Figure 1. Effect of stratification (4 ± 1°C) length on *Scirpus acutus* seed germination. Data are separated by seed origin and number of weeks to germination. Temperature regimes are pooled.

significantly ($P > 0.05$) in the Hemet or in the Denver seeds. Additionally, when non-germinated scarified and control seeds were cold stratified for 2 weeks, germination of the control seeds was improved more than that of the scarified seeds. In these investigations, scarification did not affect germination rate of seeds either before or after a cold treatment. This finding agrees with Isely (1944), who reported that scarification of *Scirpus* seeds did not improve germination.

Cold stratification was effective in breaking dormancy in *S. acutus*, and the longer the cold period (up to 12 weeks), the more completely the seeds were released from dormancy. Barton (1961) wrote that many dormant water plant seeds after-ripen in water at temperatures near or several degrees above the freezing point. When the seeds have fully after-ripened, germination may occur at the low temperature, but will certainly occur upon transfer to a higher temperature if viable. Isely's (1944) work concurs. He showed that germination percentages of eleven species of *Scirpus* improved with longer after-ripening periods in which the seeds were kept in a moist medium or in water at 2–4°C. Muenscher (1936) also found that two *Scirpus* species he tested showed little or no germination until after storage in water at 1–3° C. Additionally, the percentage of germination increased as the period of cold storage increased from 2 to 5 and then to 7 months. Barton (1961) commented on the Muenscher (1936) report that even 7 months of storage was not sufficient to fully ripen some of the seeds.

Both Denver and Hemet seeds were stimulated to germinate by long stratification treatments. However, the Hemet seeds also responded to shorter periods of stratification. More Hemet than Denver seeds were able to germinate without a cold period or with a shorter 2-to 4-week cold period under the 18/22°C temperature regime (Figure 2). Winters in Hemet, California are short; air temperature drops to or below freezing about 30 days a year (data from the National Climatic Data Center, Asheville, NC, 1994). Maguire and Heu-

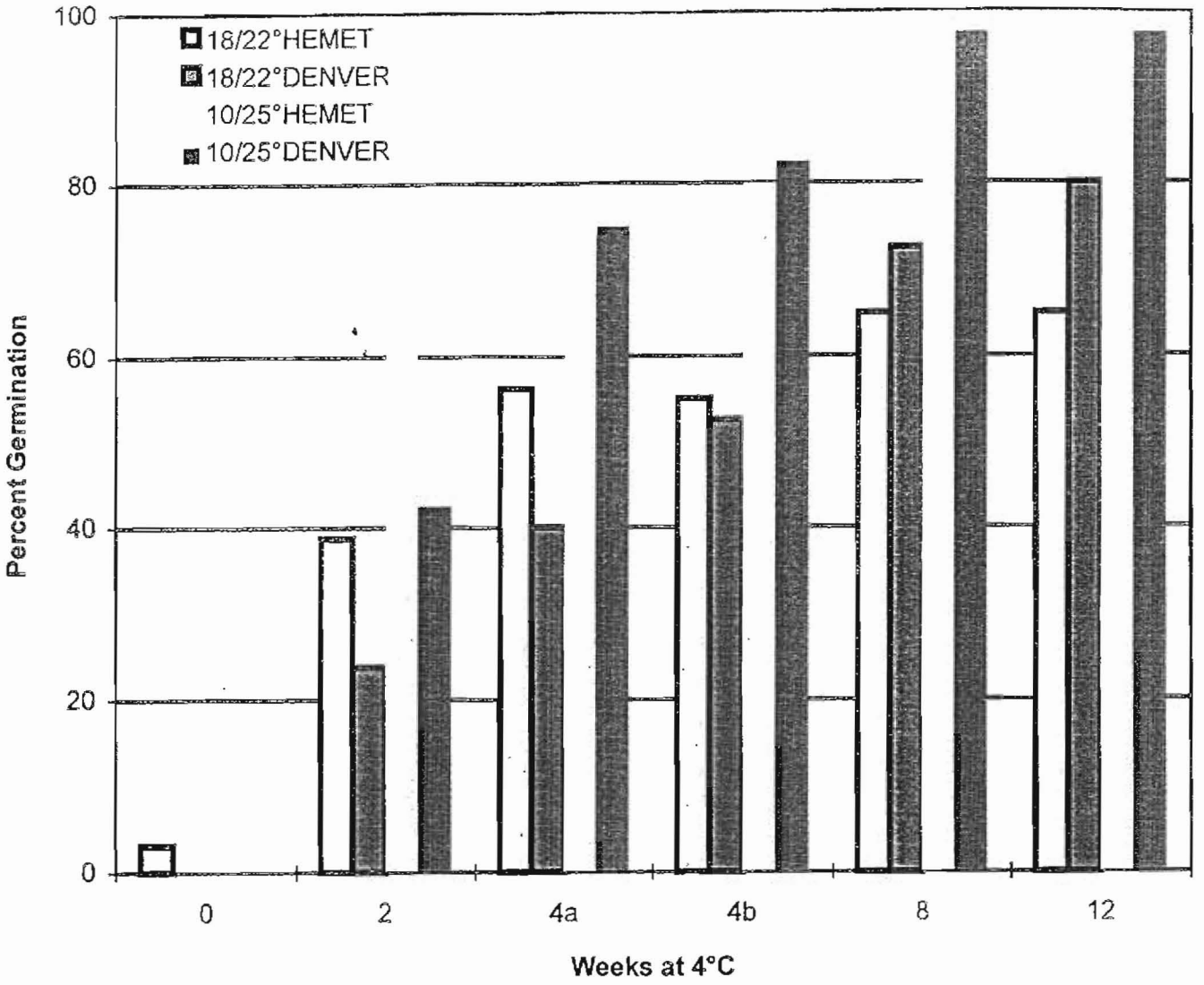

Figure 2. Effect of stratification (4 ± 1°C) length on *Scirpus acutus* seed germination. Data are the final germination results and are separated by temperature regime and seed origin.

terman (1978) stated that dormant seeds tend to be more sensitive and germinate under narrower photoperiod and thermoperiod regimes than non-dormant seeds. Perhaps the Hemet seeds were less dormant than the Denver seeds at the time of placement into the controlled chambers. The need for a moist artificial cold treatment (stratification) to break dormancy is probably related to the amount of cold treatment the seeds would normally receive before germination at the native field site. The final germination data are not significantly different between the two seed lots, but these data indicate some slight regional differences in fresh seeds.

Scirpus acutus seeds germinated to a significantly higher percentage ($P < 0.05$) in the 10/25°C temperature regime than the 18/22°C temperature regime in all of the tests (Figure 2). Although we did not examine constant temperatures for the germination period, constant temperatures and other diurnal temperature conditions were investigated by others (Muenscher 1936, Oborn 1938, Isely 1944, Harris and Marshall 1960, Shipley et al. 1989). The highest *S. acutus* germination percentage of the five previous studies was achieved by Isely (1944) in only one of his seed lots that was given a stratification period of 6 months before a period of constant illumination and a temperature regime of 30–32°C. Under these conditions, he achieved 74% germination. With the exception of a 53% germination measured by Harris and Marshall (1960) of one seed lot they left out in natural conditions (with no specific temperatures given and no replications), *S. acutus* germination in all other previous studies was less than 50%. Because germination exceeded 90% under the 15°C diurnal fluctuation of the 10/25°C temperature regime in both Denver and Hemet seeds (higher than what any other investigator achieved), the seeds are

Table 2. Percentage germination of *Scirpus acutus* seeds stratified at 4°C for the number of weeks stated, and later exposed to two diurnal temperature regimes.

Duration of Stratification (Weeks)	Temperature Regimes 18/22°C (10 h at 18°C 14 h at 22°C) a	10/25°C (10 h at 10°C 14 h at 25°C) b	Percent Difference b − a
0	2	6	4
2	31	56	25
4 a&b	50	77	27
8	69	90	21
12	73	95	22

believed to have responded favorably to the 15°C diurnal fluctuation (Berrie 1987). According to the data, the temperature regime separated by 15°C appears to be the optimal diurnal fluctuation to initiate spring germination. A similar response was observed for *Polygonum persicaria* L. (Berrie 1987) where stratification coupled with diurnal temperature fluctuation increased germination percentage quantitatively. The combined effects of stratification and the germination response of the 15°C diurnal temperature fluctuation in *S. acutus* enhanced germination by more than 20% over the 4°C diurnal temperature fluctuation of the 18/22°C temperature regime (Table 2).

The maxima of 97.5 and 92.5% germination in the Denver and Hemet seeds are greater than in any other *S. acutus* study cited previously. Therefore, *S. acutus* seeds are believed to respond optimally to the favorable environment of a stratification period of 12 weeks followed by a diurnal temperature fluctuation of 15°C, similar to the 10/25°C examined here. Manipulation of *S. acutus* seeds in the manner described would provide high germination percentages for seeding in constructed wetlands.

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