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Factors Influencing Germination of Six Wetland Cyperaceae

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Abstract: In order to determine factors which may affect the differential seed germination of six members of the Cyperaceae which occur together in newly revegetated sedge meadow, seeds were tested for germinability at three storage conditions. Seeds were also germinated at two alternating temperature regimes, 21/25° and 25/32°C. Germination of dry stored *Carex hystericina* and *C. vulpinoidea* was greater at the higher alternating temperature of 25/32°C while *Scirpus atrovirens* and *S. cyperinus* dry stored seed had a similar germination response at both alternating temperatures. Most of the moist-cool stored (stratified) seeds of the six species germinated well regardless of temperature. Seeds of these tussock-forming sedge meadow species germinate best when stratified, and higher temperatures may assist in breaking dormancy of dry stored seed. Dry-cool storage slows after ripening and seed may re-enter dormancy.

Introduction

Today, wetland mitigation policies include wetland creation or restoration as methods of ameliorating adverse impacts resulting from development. Revegetation of sites chosen for mitigation is often more cost effective if seed, rather than vegetative propagules, are used. Thus, it is important to determine appropriate seed collection and storage methodologies as well as germination requirements for wetland seeds.

Seed germination studies have been conducted in a variety of wetland species (Isely 1944, Harris & Marshall 1960, Johnson et al. 1965, Smith 1972, Thompson 1974, Kelley and Bruns 1975, Baskin and Baskin 1978, Comes et al. 1978, Keddy and Ellis 1985, Galinato and van der Valk 1986, Baskin and Baskin 1988, Larson and Stearns 1990, Shipley and Parent 1991, Larson 1993).

Only a few studies have compared the seed germination requirements of the dominant species growing within a particular wetland (Galinato and van der Valk 1986, Larson 1993). Since sedge meadows are generally

dominated by plants (Cyperaceae) of a similar growth form, knowledge of the germination requirements of such species should provide valuable insight useful for wetland restoration. This paper compares seed germination traits of six perennial tussock-forming Cyperaceae (*Carex hystericina*, *C. stipata*, *C. scoparia*, *C. vulpinoidea*, *Scirpus atrovirens*, and *S. cyperinus*), at two alternating temperature regimes. Results of my study may also assist in predicting germination requirements of related species, having similar life histories.

These six species are inhabitants of a newly revegetated peatland in southeastern Wisconsin. Of the six, *S. cyperinus* is dominant, followed in importance by *C. scoparia* with the remaining species occurring infrequently. *C. hystericina* was restricted to areas along drainage ditches and creek banks.

Methods

Seed was collected in 1989; in and near a naturally revegetated sedge meadow located within Section 27 T7N, R18E, Waukesha County, Wisconsin. The floristic composition of the sedge meadow was described earlier by Larson and Stearns (1989) and Larson (1989).

Seeds were harvested dry, and placed in paper bags, at room temperature (dry-warm). For each species, 100 freshly matured seeds were tested for ability to germinate at an alternating temperature of 21/25°C within one month of collection. At this time, some seeds of each species were transferred to two other storage conditions. Seeds were stored dry in a cool room at 4°C (dry-cool), and other seeds (except *S. cyperinus*) were stratified moist at 4°C (moist-cool). Germination data for moist-cool stored *S. cyperinus* seed was obtained from another study (Larson 1989).

Seeds were germinated in a Sherer controlled environment chamber (mode CEL 25-7HL). Two alternating temperature regimes were used: (1) 21°C dark and 25°C light (designated 21/25°C) and (2) 25°C dark and 32°C light (designated 25/32°C). Since seeds of each species have different in-field ripening times, seed age varied between five and seven months old, when the germination tests were performed.

Alternating temperature regimes were used, as prior studies have found alternating temperatures effective in producing germination (Thompson 1974, Thompson and Grime 1983). Since most germination studies on Cyperaceae have found light to be essential (Isely 1944, Bliss 1958, Johnson et al. 1965, Baskin and Baskin 1988), a 14-hour light, 10-hour dark regime was used. Daytime light (supplied by cool white florescent tubes) levels were 200 $\mu\text{mol M}^{-2} \text{S}^{-1}$ at seed level.

For germination, 50 seeds/petri dish (except for *S. cyperinus* and *S. atrovirens*) were placed on filter paper (Whatman No. 1) moistened with 5-6ml of distilled water. *Scirpus cyperinus* and *S. atrovirens* seed presents a special problem in that the seeds are very small and *S. cyperinus* seed easily become entangled in the bristles. To avoid damage to the seed, samples believed to contain 100-300 seeds were used; the exact number was determined at the end of a test. For each species, four replicate petri dishes were used for each storage condition and temperature regime. Dishes were watered every other day using distilled water. Initial germination counts were made when the first shoot and radicle appeared outside a perigynium and every 3-5 days thereafter, until the experiment was terminated at 21 days after placing seeds in petri dishes.

For each species seed germination data was analyzed in two ways. Log likelihood ratio Chi-squares were calculated to test if germination rates were affected by: 1) test temperature regime (21/25° vs 25/32°C, 2x2 contingency table); 2) storage condition (dry-warm, dry-cool, or moist-cool, 2x3 contingency table); or 3) storage condition with test temperature held constant (two, 2x3 contingency tables, one for each test temperature). Seed germination data was also converted into percentages and differences between storage conditions at a given temperature regime were analyzed using one-way analysis of variance. When variances were heterogenous, the Games and Howell test for equality of means was used (Sokal and Rohlf 1981). Differences were considered significant at the 0.05 level.

Results

Freshly harvested seeds of *C. stipata* and *C. scoparia* gave 60% and 1% germination, respectively. The freshly harvested seeds of the remaining four species did not germinate.

C. hystericina, *C. vulpinoidea*, *S. atrovirens*, and *S. cyperinus* germination percentages were highest when seed was stored under moist-cool conditions, regardless of germination temperature regime (Tables 1 and 2). Additionally, *C. hystericina*, and *C. vulpinoidea* seed stored dry (warm or cool) had higher germination when tested at 25/32° than 21/25°C (Tables 1, 2 and 3).

Moist-cool *C. scoparia* seed had significantly lower germination (45% and 49%) than either the dry-warm or dry-cool seed, with the dry-cool seed test at 25/32°C being an exception (Tables 1 and 2). *C. scoparia* dry-warm stored seed germinated equally well at both temperatures while dry-cool stored seed had higher percentage germination when tested at 21/25°C than at 25/32°C (Table 3).

Seeds of *S. cyperinus* required stratification (Tables 1 and 2). In an earlier study moist-cool *S. cyperinus* seed had no consistent pattern of increasing germination with length of stratification (Larson 1989). Thus, it appears that the

higher alternating temperature of 25/32°C may be responsible for the increased germination (94%) as compared to the lower alternating temperatures of 21/25°C (75%), and not the additional six months of moist storage. *S. atrovirens* seed did not require stratification, but had improved germination when moist stratified regardless of germination temperature. Germination temperature did not have a significant effect on germination of either *Scirpus atrovirens* or *Scirpus cyperinus* (Table 3). *C. stipata* seed from all three storage conditions germinate almost equally well, regardless of germination temperature regime (Tables 1, 2 and 3).

Of the seeds stored moist-cool and tested at either 21/25° or 25/32°C, only *C. scoparia* did not exceed 50% germination. For all six species, the mean germination percentages were higher for stratified (79%) than for non-stratified seeds (53% dry-warm and 42% dry-cool). Alternating temperatures of 25/32°C resulted in a higher mean germination (64%) than 21/25°C (53%).

Table 1. Percent seed germination of six wetland emergent species tested at 21/25°C. Standard deviations in parentheses. Percentages followed by the same letter are not significantly different (alpha 0.05) across columns. n = 200 for each species and storage treatment except *Scirpus cyperinus* and *S. atrovirens*. For *S. cyperinus*, dry-warm, n = 331; dry-cool, n = 311; moist-cool, n = 993; *S. atrovirens*, dry-warm, n = 321; dry-cool, n = 459; moist-cool, n = 764.

Species	Dry-warm	Dry-cool	Moist-cool
<i>Carex hystericina</i>	18a (5.9)	3b (3.8)	81c (5.8)
<i>Carex scoparia</i>	79a (6.8)	79a (6.6)	45b(10.6)
<i>Carex stipata</i>	88a (6.0)	82a (7.6)	79a (7.6)
<i>Carex vulpinoidea</i>	21a (8.9)	8b (4.1)	85c (6.2)
<i>Scirpus atrovirens</i>	59a (7.7)	51a (3.3)	96b (0.9)
<i>Scirpus cyperinus</i>	0a (0.0)	0a (0.0)	75b*(9.0)

*Seed was six months old when tested (Data from Larson 1989).

Table 2. Percent seed germination of six wetland emergent species at 25/32°C. Standard deviations in parentheses. Percentages followed by the same letter are not significantly different (alpha 0.05) across columns. n =200 for each species and storage treatment except *S. cyperinus* and *S. Atrovirens*. For *S. cyperinus* dry-warm, n=255; dry-cool, n = 326; moist-cool, n = 569. For *S. atrovirens* dry-warm, n = 446; dry-cool, n = 562; moist-cool, n = 668.

Species	Dry-warm	Dry-cool	Moist-cool
<i>Carex hystericina</i>	74a (10.3)	30b (5.0)	80c (9.3)
<i>Carex scoparia</i>	74a (10.3)	39b (11.8)	49b (16.7)
<i>Carex stipata</i>	86a (6.2)	77a (10.1)	82a (4.3)
<i>Carex vulpinoidea</i>	82a (5.0)	63b (6.2)	87a (6.2)
<i>Scirpus atrovirens</i>	54a (12.1)	67a (16.3)	98b (1.8)
<i>Scirpus cyperinus</i>	1a (1.2)	<1a (1.0)	94b* (1.7)

*Seed was 12 months old when tested (data by Larson 1989).

Table 3. Log likelihood ratio Chi-square statistics for tests of independence of seed germination and storage and test conditions. See Tables 1 and 2 for sample sizes.

Species	Test Temperature 1df	Storage Cond. 2df	Storage Cond. Tested at	
			21/25°C 2df	25/32°C 2df
<i>Carex hystericina</i>	91.0***	356.0***	332.3***	127.0***
<i>Carex scoparia</i>	23.6***	76.0***	68.8***	54.0***
<i>Carex stipata</i>	0.37 ns	9.50**	6.15*	5.44 ns
<i>Carex vulpinoidea</i>	196.1***	236.7***	310.7***	35.6***
<i>Scirpus atrovirens</i>	0.51 ns	769.7***	410.5***	393.3***
<i>Scirpus cyperinus</i>	0.53 ns	2304***	1137***	1275***

ns, not significant, $p > 0.05$; *, $0.01 < p < 0.05$; **, $0.001 < p < 0.01$; ***, < 0.001 .

Discussion

The germination requirements of *C. scoparia*, *S. atrovirens*, and *S. cyperinus* have been studied previously (Isley 1944, Larson 1989, Larson and Stearns 1990, Shipley and Parent 1991, Larson 1993). Despite the different temperature regimes and storage conditions used in these and other wetland seed germination studies, results from my study are generally consistent with those previously described. That is, seed is initially dormant and alternating high temperatures and stratification increase germination, while dry storage (warm or cool) decreased germination. There were two exceptions to this pattern, *C. stipata* was not initially dormant and *C. scoparia* seed germinated poorly after being stored moist-cool.

Dormant seeds usually become non-dormant only at specific temperatures (Baskin and Baskin 1988). Since the fresh seeds in my study were tested at only one temperature regime (21/25°C), it is not possible to determine if five of the species were completely dormant. *C. scoparia* and *S. cyperinus* seed have previously been found to be initially dormant at several alternating temperature regimes, suggesting that these species are initially dormant (Larson 1989, Larson and Stearns 1990, Larson 1993).

Prior studies involving the germination requirements of various Cyperaceae found no single treatment was effective for all species (Isely 1944, Johnson et al. 1965, Baskin and Baskin 1988, Larson 1989, Larson 1993). In my study, moist-cool storage was effective for at least 50% germination of all but *C. scoparia*, regardless of germination temperature regime.

Comes et al. (1978) also found that a number of wetland species required moist-cool storage before seeds would germinate, which also appears to be a requirement for *S. cyperinus*. Other species (i.e., *C. scoparia* and *S. atrovirens*) that were initially dormant apparently after-ripen under a variety of storage conditions.

Johnson et al. (1965) found periods of stratification exceeding 90 days had little added effect on the germination of some Wyoming sedges, and for some, it may have actually inhibited germination. This may be the case for *C. scoparia* as germination was reduced by six months of stratification. This was also observed in one previous study of *C. scoparia* (Larson and Stearns 1990), but not in another study (Larson 1993).

With the exception of *S. cyperinus*, stratified seed germinated equally well at both temperature regimes. This lack of a specific temperature requirement for stratified seeds has been observed by others (Galinato and van der

Valk 1986, Baskin and Baskin 1988). Higher germination of dry-cool stored seed at 25/32°C (i.e., *C. hystericina* and *C. vulpinoidea*) may indicate that dormancy may be broken by an alternating higher temperature.

C. stipata and *S. atrovirens* appear to belong to a group of species that germinate at any time during the growing season, when light, temperature, and moisture are sufficient.

Plants of a particular habitat may share or display more than one germination pattern (Angevine and Chabot 1979). However, two habitats, mesic deciduous forests and mud flats, have high percentages of species with the same or similar seed temperature responses (Baskin and Baskin 1988). In my sedge study, *C. hystericina* and *C. vulpinoidea* were most similar in germination response to the various storage and temperature regimes. Both of these species have been found in mud flat conditions in this study. In another study, two *Bidens* species typical of mud flats had a similar germination response as *C. hystericina* and *C. vulpinoidea* (Larson 1993).

Dormancy breaking and germination requirements have been found not to be phylogenetically constrained (Baskin and Baskin 1988). Within some families there is a tendency for a given type of seed-temperature response to be important. In my study, most of the Cyperaceae had equal or greater germination when tested at 25/32°C than at 21/25°C. Further examination of the seed germination requirements of other Cyperaceae, especially those found in sedge meadows, is still necessary.

My results suggest that seeds of three tussock-forming sedge meadow species germinate best when stratified, and that a higher germination temperature assists in breaking dormancy. Dry-cool storage slows after-ripening and seed may re-enter dormancy. The variety of germination responses of these cyperaceae may enable them to establish successfully in sedge meadows where seasonal, yearly, and microenvironmental conditions fluctuate.

Species whose seeds can germinate under a broad range of environmental conditions are excellent candidates for use in wetland mitigations. Since most wetland cyperaceae appear to require or show improved germination when stratified, the timing of seeding is crucial to the success of any wetland mitigation project.

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