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Germination Requirements of 32 Native Washington Prairie Species

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ABSTRACT

Ecological restoration has become a critical component of preservation and management of prairie remnants in Washington State. Active restoration projects typically require production of large quantities of native plant materials, and large-scale plant production requires provision of appropriate germination conditions for many species of native plants. We tested 31 native Washington prairie plant species for germination requirements including after-ripening and combinations of stratification and germination temperature. 24 of the species tested germinated in sufficient numbers for analysis; one and two-way fixed-effects ANOVAs were used to determine treatment and interaction effects. Partial tests were conducted when less than 2000 seeds of a species were available (collected). Species that showed low germination under all treatments were tested for viability with tetrazolium. Study species exhibited a variety of germination strategies, and, for presentation purposes, have been grouped based on pretreatment yielding the highest germination rate. Although the effects of germination temperature were often not statistically significant, we observed generally higher seedling survival and vigor under cool germination temperatures.

INTRODUCTION

A vast majority of prairie habitat in the Puget Sound lowlands has been lost to development, fire suppression, and other anthropogenic disturbance. Remaining prairie habitats are highly susceptible to encroachment of invasive plants and additional fragmentation. In an attempt to counteract degradation and invasion, intensive restoration strategies are being developed and practiced on remnant Puget lowland prairies.

Active restoration of prairie habitats requires producing, planting, and maintaining large quantities of native plant materials. The extensive time, money and resources which managers commit to seedling production and installation make efficient propagation of plants for revegetation projects a necessity. This study addressed a critical component of plant propagation, determination of seed germination requirements of species used in active restoration.

We tested germination pretreatment requirements of 31 native Washington prairie species. The mild, wet winter-dry summer climate west of the Cascades made cold-moist stratification a logical choice of pretreatments for plants of this region. A literature review and anecdotal evidence from the region suggested that seeds of Washington prairie species are likely to respond to after-ripening, cold, moist stratification and temperature variation treatments.

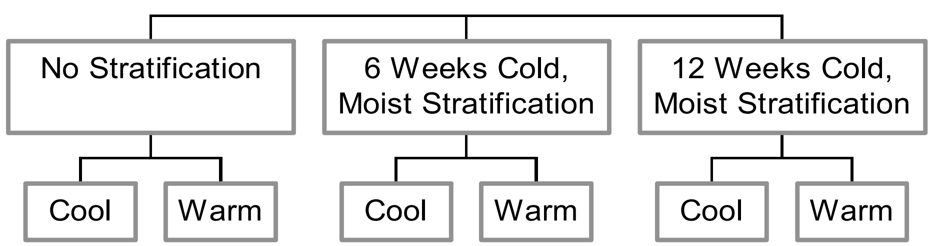
METHODS

Seed collection and storage

Seeds of 31 Native Washington Prairie plants were collected by the Washington Nature Conservancy between June and October, 1995 on the Fort Lewis Army Base, near Tacoma, Washington. Between 30 and 20,000 seeds of each species were collected. Seeds were stored in paper bags at room temperature until they were delivered to the University of Washington. Seeds were then stored under refrigeration. The unrefrigerated storage period was equivalent to 3-6 months after-ripening. Germination testing took place at the University of Washington, Seattle in January through May, 1996.

Experimental design and statistical analysis

Three replicates of 100 seeds were tested for each treatment. Replicates of 100 seeds were chosen from single seed batch, where batch refers to time and general location of collection as recorded by the Nature Conservancy. A full factorial design with two temperature conditions and three stratification regimes was used; a total of 6 treatments for each species. Full 2-factor tests consisted of the following:



One and two-way fixed-effects ANOVAs were used to determine significant treatments and interaction effects. Because variance in ecological studies is generally high, we used a 10% significance (alpha) level (personal communication, Professor Loveday Conquest, Center for Quantitative Science). One-way ANOVAs were calculated when not enough seeds were available for full treatments (i.e. only one treatment effect was tested).

Tetrazolium (viability) testing was performed on species that showed low germination under all treatments. Partial tests were conducted on species for which less than 2000 seeds were available. Standard methods of germination testing, including sample size and replicate number, were based on standard plant propagation techniques (Hartmann et al. 1990, personal communication, Barbara Selemon, plant propagator, University of Washington

Arboretum).

Pretreatments, germination and viability

Seeds were stratified in moist, sterile, inorganic soil mix under refrigeration at 2-6^{o} C for 6 or 12 weeks (the third stratification treatment was no stratification). Cool temperature treatments were germinated at ambient (outside) temperature in a plastic-covered frame to protect plantings from predation and wind (48-65^{o} C). Warm treatments were germinated in a greenhouse with regulated temperatures (65-70 night, 70-85 day). Seeds were sown on approximately 3 cm depth packed soil in standard flats, and covered with approximately .5 cm soil for germination. Flats were checked daily for soil moisture and watered as needed (2 to 7 times per week). Flats were monitored (germinants counted) 2-3 times weekly for four weeks after planting. Tetrazolium (2,3,5-Triphenyl-2H-tetrazolium Chloride) viability testing was performed on species with low germination rates. If available, 4 replicates of 25 seeds each (replicates from different seed batches) of each species were tested for viability.

RESULTS

The group of Native Washington prairie species we tested showed a variety of germination strategies. Almost all (22 of 30) responded well to either 6 or 12-week stratification. Several species responded to 6-week stratification but were damaged by 12-week stratification *(Lomatium nudicaule*, *Lomatium utriculatum, Lupinus albicaulis, Ranunculus occidentalis*, and *Zigadenus venenosus)*. Table 1 contains a summary of treatment and interaction effects for each species tested. Results of tetrazolium viability testing are summarized in Table 2. Table 3 contains germination data for all species under all test conditions.

TABLE 1.

Most effective treatment	Maximum Germination (%)	Stratification P	Temperature P	Interaction P
No stratification, warm germination				
Cerastium arvense	82.7	0	0	0.001
6 weeks of cold, moist stratification, temperature indifferent				
Aster curtus	8.7	0.001	0.258	0.49
Eriophyllum lanatum	31	0	0.505	0.671
Hieracium cynoglossoides	42	0.039	0.044	0.843
Lomatium utriculatum	58.7	0	0.245	0.262
Ranunculus occidentalis	52	0.058	0.772	0.289
Solidago spathulata	12.3	0.107	0.877	0.488
12 weeks of cold, moist stratification (temperature indifferent)				
Camassia quamash	84.5	0	0.128	0.255
Marah oregana *	45	0.003	not tested	
Lomatium triternatum	6.7	0.051	0.876	0.975
Silene scouleri *	37	0.062	not tested	
Benefit from either or both stratification regimes				
Campanula rotundifolia	80	0	0.128	0.255
Castilleja hispida *	2.3	0.06	0.26	0.26
Fritillaria lanceolata *	7.3			
Panicum scibnerianum *	13.7	0.204	0.074	0.326
Interaction between stratification and germination temperature (i.e. a combination of stratification and germination temperature yields highest germination).				
Balsamorhiza deltoidea	30.7	0.002	0.005	0.012
Delphinium nuttallii	3.3	0.168	0.224	0.082
Dodecatheon hendersonii	59	0	0	0.004
Dodecatheon pulchellum	44	0	0.001	0

	Maximum Germination (%)	Stratification P	Temperature P	Interaction P
Interaction between stratification and germination temperature (i.e. a combination of stratification and germination temperature yields highest germination).				
Erigeron speciosus	36	0	0.041	0.044
Luzula campestris	58	0	0.001	0.032
Lupinus albicaulis	31.7	0	0.009	0.016
Saxifraga integrifolia	25.3	0	0	0
Zigadenus venenosus	72.3	0	0	0
Equal germination in all treatments				
Antennaria neglecta	9.7	0.538	0.577	0.365
Insignificant germination (<1%) under all treatments				
Brodiaea coronaria *		No pretreatment, 0/160 seed germination		
Brodiaea hyacinthina *		No pretreatment, 0/85 seed germination		
Lomatium nudicaule *		No pretreatment, 0/600 seed germination		
Lupinus lepidus *		No pretreatment, 0/32 seed germination		
Panicum occidentalis		Full treatments, 0/1200 germination		
Sisyrinchium angustifolium		Full treatments, 2/1200 seed germination		
Viola adunca *		No pretreatment, 0/131 seed germination		

TABLE 2.

Tetrazolium testing

Species	% Viable
Apocynum androsaemifolium	10.1
Aster curtis	13.1
Balsamorhiza deltoides	52.0
Castilleja hispida	15.0
Delphinium nuttallii	77.5
Fritallaria lanceolata	3.4
Lomatium nudicaule	67.0
Lomatium triternatum	63.0
Panicum occidentalis	0.0
Sisyrinchium angustifolium	3.0
Zigadenus venenosus	76.2

TABLE 3.

Species		No Stratification	days until germination	6 Weeks	days until germination	12 Weeks	days until germination
Antennaria neglecta	warm	4.7%	20	6.3%	34	1.3%	12
	cold	0.3%	34	8.3%	48	9.7%	12
Apocynum androsaemifolium	warm	0.0%		0.0%		0.0%	
	cold	0.0%		0.0%		2.0%	30
Brodiaea coronaria *	No germination						
Brodiaea hyacinthina *	No germination						
Aster curtus	warm	0.7%	20	6.0%	11	2.7%	10
	cold	0.0%	20	8.7%	13	3.3%	12
Balsamorhiza deltoidea	warm	1.0%	32	6.0%	11	10.3%	5
	cold	0.0%		30.7%	18	15.3%	5
Castilleja hispida	warm	0.0%		0.0%		1.0%	16
	cold	0.0%		0.0%		2.3%	20
Camassia quamash	warm	0.0%		39.7%	8	72.3%	5
	cold	0.0%		47.0%	22	84.5%	5
Campanula rotundafolia	warm	54.0%	31	72.3%	6	26.3%	12
	cold	0.0%		66.7%	22	80.0%	12
Cerastium arvense	warm	82.7%	9	61.3%	6	2.7%	13
	cold	48.0%	18	21.3%	8	4.0%	13
Delphinium nuttallii	warm	0.3%	23	0.3%	15	0.7%	10
	cold	0.0%		0.0%		3.3%	16
Dodecatheon hendersonii	warm	0.0%		3.7%	11	14.0%	10
	cold	0.0%		37.3%	25	59.0%	5
Dodecatheon pulchellum	warm	0.0%		14.7%	30	0.0%	
	cold	0.0%		44.0%	32	0.3%	23
Eriophyllum lanatum	warm	5.0%	9	31.0%	6	3.0%	12
	cold	10.0%	17	29.3%	15	6.0%	19

TABLE 3.

Erigeron speciosus	warm	16.3%	7	36.0%	6	6.0%	9
	cold	3.7%	19	13.0%	15	4.3%	23
Fritillaria lanceolata *	warm	0%		0.0%		6.0%	19
	cold	0%		0.0%		7.3%	12
Hieracium cynoglossoides	warm	12.5%	8	42.0%	6	14.7%	16
	cold	0.5%	22	21.0%	18	5.3%	30
Lomatium triternatum	warm	0.0%		0.0%		5.7%	5
	cold	0.0%		0.0%		6.7%	5
Lomatium utriculatum	warm	0.0%		53.0%	6	0.0%	
	cold	0.0%		58.7%	13	0.0%	
Lomatium nudicaule *	warm	0.0%		0.0%		0.0%	
	cold	0.0%		0.0%		0.0%	
Lupinus lepidus *	No germination						
Luzula campestris	warm	58.0%	14	49.7%	4	0.0%	
	cold	32.0%	22	23.7%	10	0.0%	
Lupinus albicaulis	warm	17.0%	7	31.7%	2	0.0%	
	cold	4.3%	25	29.3%	2	0.0%	
Marah oregana *	warm	0.0%		0.0%		45.0%	
Panicum scibnerianum *	warm	4.7%	24	0.0%		13.7%	10
	cold	0.0%		0.0%		1.3%	19
Panicum occidentalis	warm	0.0%		0.0%		0.0%	
	cold	0.0%		0.0%		0.0%	
Potentilla gracile	warm	0.0%		11.7%	8	0.0%	
	cold	0.0%		20.7%	18	0.0%	
Prunella vulgaris	warm	16.7%	9		0	0.0%	
	cold	4.7%	13		0	0.0%	
Ranunculus occidentalis	warm	42.7%	16	52.0%	22	0.0%	0
	cold	45.0%	24	48.0%	29	0.0%	0

TABLE 3.

Silene scouleri *	warm	0.0%	0	0.0%	0	37.0%	20
	cold	0.0%	0	0.0%	0	0.0%	0
Saxifraga integrifolia	warm	0.0%	0	0.0%	0	0.7%	9
	cold	0.0%	0	25.3%	29	4.7%	16
Sisyrinchium angustifolium	warm	0.0%	0	0.8%	13	0.0%	0
	cold	0.0%	0	0.0%	0	0.0%	0
Solidago spathulata	warm	6.7%	14	9.3%	8	1.0%	10
	cold	0.0%	0	12.3%	18	2.3%	12
Viola adunca *	No germination						
Zigadenus venenosus	warm	0.0%	0	14.7%	15	0.0%	0
	cold	0.0%	0	72.3%	29	1.3%	26

*** Species for which less than 2000 seeds were available (limited to partial testing)**

DISCUSSION

Prairie plant species found at Ft. Lewis are generally not specialists and most range widely across the Western United States and Canada. Many Puget Sound prairie species are "weedy" in habit. Not surprisingly, a variety of reproductive strategies are represented in this plant community. Many species germinate under a variety of conditions but appear to do best under one particular regime. No one strategy or germination requirement appears to dominate the group of species we tested. In general, however, the species tested responded best to some period of stratification and cool germination temperatures.

Several species tested, such as *Frittalaria lanceolata*, *Delphinium nuttallii*, and *Panicum occidentalis* responded poorly to all treatments, and will require additional testing for specific requirements. The results of tetrazolium testing indicate different reasons for low germination in these species; *F. lanceolata* shows very low viability (3.3%) of seeds but a relatively high proportion of viable seeds germinated (7%). This apparent decrease in viability suggests detrimental effects of after-ripening, but differences are probably not statistically significant. *D. nuttallii* exhibited low germination under all treatments (maximum 3.3%) but very high seed viability (77.5%). We were unable to determine appropriate germination cues for *D. nuttallii* in this study. *P. occidentalis* exhibited 0% germination and 0% viability. Viability of *P. occidentalis* (and other species) may be decreased by after-ripening. 1995 may also have been a poor year of seed production for many species. Multi-year tests that control for effects of after-ripening are required to determine the cause of low germination in several of these study species.

Research Recommendations

Seed collection for future research
Seeds tested in this research were collected from 3-6 months before treatments were started. Germination treatments should be conducted immediately after seed collection to establish after-ripening requirements and to minimize loss of viability for species which do nor require after-ripening. Seed should be stored under refrigeration immediately after collection.

* Field testing to determine germination timing of important species (spring, summer or fall germination); appropriate timing of plantings and seedings will be crucial for successful restoration projects.

* Seed bank testing to determine natural densities of desired species' seeds.

* Comparison/cost-benefit analysis of survival of transplanted plants to direct seeding.

* Testing of potential seed mixes for direct sowing projects.

* Additional germination testing of special interest species, and species with low germination
and determination of natural variation of seed viability between sites.

Greenhouse vs. Outside Germination

Overall, propagation of native prairie plants under ambient (outside) conditions in Washington appears to provide greater benefits than greenhouse propagation. Outdoor propagation is less expensive than greenhouse propagation, provides a more "natural" environment for plants physiological acclimatization, and appears to enhance germination of many difficult-to-grow species such as *Balsamorhiza deltoidea*, *Dodecatheon sp*., and *Saxifraga integrifolia*. Drawbacks of outdoor propagation include the effects of predation by birds, insects and small mammals. During the course of this experiment germination was observed to take generally longer in cooler conditions (2 days to 4 weeks longer), although plants raised in the greenhouse often appeared to be less "healthy" than plants grown outside. Desiccation may have contributed significantly to "warm" effects.

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LITERATURE CITED

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