The Effect of Cold Stratification on the Germination of Grassland Seeds

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Abstract

One factor that must be considered when reconstructing a prairie is how the prairie seeds being planted need to be prepared for germination. The probability of successful germination of these seeds is dependent on many factors, including exposure to cold temperatures for a prolonged period of time. To explore this idea, we collected seeds from 13 species of forbs and grasses, both native and non-native, and stored them at various temperatures for several weeks. We predicted that the germination of the seeds of native grassland species would be enhanced by cold stratification, while non-natives (especially forbs common to flower gardens) could be negatively affected by cold stratification, especially if they are native to an area with less-extreme winters. We also predicted that seeds stored at -80°C (a temperature much lower than they would experience in nature) would respond negatively to the treatment and be unable to germinate. We found that four species (Sow Thistle, Penstemon, Queen Anne's Lace, and Yellow Foxtail) were significantly affected by the seeds being chilled or frozen, with non-native sow thistle and yellow foxtail responding negatively to being frozen and native foxglove penstemon germinating best at -80°C.

Introduction

Stratification of seeds is a process of treating a seed in dormancy with simulated natural situations to end its dormancy and allow for germination (Boddy et. al 2013). For different species, there are different requirements to break a seed out of dormancy. One of the requirements that affect native plant species in the Midwest is whether the seeds experience a prolonged freeze. Native plants typically drop their seeds in late fall, which allows them to go through a winter freeze and then germinate in the spring when they warm up (Christensen & Landers 1966). By doing an experiment on seed stratification, we can understand better how to treat collected seeds to be used for prairie reconstruction and what time of year to best seed native prairies.

The BIO205 Ecology class had the opportunity to study the effects of different temperature on thirteen species of forbs and grasses, both native and non-native. This was done to determine if a winter freeze would benefit the germination of seeds from any of the species selected. We hypothesized that the chilling and freezing of native prairie seeds would increase the germination probability. We predicted that 3°C and -10°C would have the highest germination rate and that 20°C and -80°C would have the lowest. We believed that 20°C would not shift the seeds out of dormancy and that -80°C would result in the seeds dying from being exposed to such an unnaturally cold temperature. We also anticipated that we might find a difference between the species native to the Midwest and seeds of non-natives such as marigolds (a sub-tropical species) and Queen Anne's Lace (native to the Nile River valley).

	20°C	3°C	-10°C	-80°C
Big Bluestem	7/72 (9.7%)	11/53 (21%)	17/82 (21%)	
Larkspur		77/116 (66%)	125/189 (66%)	
Prairie Blazing Star		4/59 (6.8%)	1/59 (1.7%)	2/40 (5%)
Purple Coneflower	1/20 (5%)	1/42 (2.4%)	2/45 (4.4%)	
Prairie Coneflower	10/40 (25%)	5/40 (13%)	7/40 (18%)	13/40 (33%)*
Spiked Speedwell	6/88 (6.8%)	12/116 (10%)	4/127 (3.1%)	
Foxflove Penstemon	5/44 (11%)	3/55 (5.5%)	5/41 (12%)	16/45 (36%)
Partridge Pea	1/69 (1.4%)	2/63 (3.2%)	1/39 (2.6%)	1/40 (2.5%)
Yellow Foxtail #	44/55 (80%)*	9/44 (20%)	20/69 (29%)	
Curly Dock #	17/60 (28%)	11/41 (27%)	28/60 (47%)	
Queen Anne's Lace #	22/160 (14%)*	7/175 (4%)	21/150 (14%)*	11/77 (14%)*
Marigold #		45/85 (53%)	32/80 (40%)	
Sow Thistle #	28/60 (47%)	34/40 (85%)*	31/60 (52%)	

Table 1. Germination results. Proportions and percentages of seeds germinated are shown. Asterisks (*) indicate significantly higher germination for these trials. Hashtags (#) indicate non-native species.

A)

B)

C)

Figure A. The Nature Conservancy's Glacier Hills Preserve in Buena Vista County. **Figure B.** Placing seeds in Petri dishes. **Figure C.** Germination experiment in progress

Results

We performed chi-square tests for all of the thirteen species to determine if the differences in germination between treatments were significant (Table 1). We found that there were significant differences in four species: Sow Thistle ($p<0.0003$; highest germination at 3°C), Foxglove Penstemon ($p<0.0003$; highest at -80°C), Queen Anne's Lace ($p<0.006$; lowest at 3°C), and Yellow Foxtail ($p<0.00001$; highest at 20°C). The other nine species showed no significant difference in germination between treatments.

References

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Materials and Methods

Seeds for this research were collected on four different dates and locations. Larkspur, Sow Thistle, Prairie Blazing Star, Marigold, and Spiked Speedwell seeds were collected on September 1, 2021, from Dr. Tracy's flower beds in Orange City. Seeds of the Purple Coneflower and Foxglove Penstemon were collected on the Northwestern College campus in Orange City on September 7, 2021. Seeds of Curly Dock and Queen Anne's Lace were collected from The Nature Conservancy's Glacial Hills Preserve in Buena Vista County on September 13, 2021 (Figure A). The seeds of Big Bluestem, Partridge Pea, Prairie Coneflower, and Yellow Foxtail were collected from the Trinity Church Prairie in Orange City on September 14, 2021.

Once all the seeds were collected, we removed the chaff and placed the seeds into envelops. The number of seeds collected dictated the number of temperatures that would be tested for the species. Seeds stored at 20°C were left in a drawer in lab throughout the temperature procedure. The seeds for 3°C, -10°C, and -80°C were placed in a refrigerator at 3°C for seven days, after which time the -10°C and -80°C envelopes were moved to their respective temperatures. We did this gradual cooling to reduce the chance of the seeds experiencing a freeze shock. We left all seeds at their final temperatures for 31 days, after which time we stored all seeds at 20°C for 21 days. We then removed the seeds from their envelopes and placed between two filter papers within petri dishes (Figure B). We treated all seeds with Daconil Fungicide and placed all petri dishes vertically in containers with water in the bottom that allowed for the filter papers to stay moist without the seeds being submerged in water (Figure C). After 21 days, we recorded the number of germinated and ungerminated seeds in each dish.

Discussion

We performed this experiment to determine whether the seeds of various species of forbs and grasses respond positively to cold stratification. We found that four of the species we studied responded differently to being stored at different temperatures. We had hypothesized that the freezing of seeds would increase germination rate, especially among native species. However, only foxglove penstemon responded positively to freezing, while the other native species showed no differential response to different storage temperatures. We conclude that of the native species studied, only Penstemon would benefit from being cold stratified if seeds collected in the fall are planted the following year. We also conclude that cold winter temperatures should not be expected to prevent the kill the seeds of marigolds or the various invasive non-natives that we studied and given the fact that the seeds of Queen Anne's Lace even germinated after being stored at -80°C, we feel that its invasion into the Arctic will not be limited by the sensitivity of seeds to cold temperatures. Our experiment could have been improved if we had collected enough seeds to test all 4 temperatures for all of the species we tested. Because of having an insufficient number of seeds, we could not perform the -80°C treatment on the other non-native species tested and thus cannot draw conclusions on those species' ability to invade colder environments.

Freezing is not the only way to stratify seeds. Some seeds need other environmental factors to break their dormancy, including the right amount of moisture, light, high temperatures, certain chemical actions (such as making their way through an animal's digestive system), and even fire (Pedrini & Dixon 2020). For the species that did not have a higher germination after freezing there could be other ways that the seed needs to stratified. This demonstrates to us the complexity of nature and how difficult it is to replicate and study that complexity in the lab.