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Arctic Seed Sterilization and Germination

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Simone S. Whitecloud

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Arctic Seed Sterilization and Germination

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Abstract

We conducted growth chamber experiments to overcome challenges of native seed germination relating to disease and germination time. We selected five northern species, *Eriophorum vaginatum*, *E. virginicum*, *Anemone patens* var. *multifida*, *Polemonium reptans*, and *Senecio congestus*, for their native ranges and commercial-nursery availability. Recommended stratification time for each species was either unknown or a minimum of 60 days. Seeds were sterilized with 70% ethanol, 10% hydrogen peroxide, or UVC light to identify which method most effectively prevented pathogen infection. To determine if stratification time could be reduced, seeds underwent a 30-day cold, moist stratification. We tested which growth medium was most conducive to germination of the sterilized, stratified seeds: filter paper or sterilized potting soil. In a separate experiment, we tested if three different levels of gibberellic acid (GA₃; 0, 500, and 1000 ppm) could reduce stratification time to 15 days. The 70% ethanol was effective in a seed surface sterilization; an average of 84% of all seeds for all species treated showed no contamination. Germination following a 30-day cold, moist stratification was unsuccessful for most species tested in both growth media. Here, 1000 ppm GA₃ with a 15-day cold, moist stratification showed considerable success with *P. reptans*.

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Preface

This study was conducted for the US Army Corps of Engineers under PE 0603119A, Project B03, “Army Visual and Tactical Arctic Reconnaissance.”

The work was performed by the Biogeochemical Sciences Branch of the Research and Engineering Division, US Army Engineer Research and Development Center–Cold Regions Research and Engineering Laboratory (ERDC-CRREL). At the time of publication, Mr. Nathan J. Lamie was branch chief; and Dr. John Weatherly was acting division chief. The acting deputy director of ERDC-CRREL was Dr. Ivan P. Beckman, and the director was Dr. Joseph L. Corriveau

COL Christian Patterson was commander of ERDC, and Dr. David W. Pittman was the director.

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1 Introduction

1.1 Background

Cultivating native plants in greenhouse conditions is challenging. Native seeds often have inherent dormancy that needs a very specific set of environmental conditions to be broken. Native seeds also may exhibit nonuniform emergence; and pathogenic microorganisms, such as bacteria or fungi, on the seed surface can cause seed rot (Luna et al. 2009). They can take weeks to germinate; and once established, more time is needed to reach the desired growth stage for sampling. The challenges of dormancy, disease, and the time needed for germination and establishment must be overcome for successful plant production in a time-sensitive research setting. We selected five cold-hardy species, including both monocots and dicots, with native ranges throughout the northern continental United States and Canada (Table 1). Three of the species, *Eriophorum vaginatum*, *Senecio congestus*, and *Anemone patens* var. *multifida*, extend farther north into Alaska and the Arctic region (USDA, NRCS 2023).

Breaking seed dormancy can be accomplished using a stratification technique to mimic their natural environmental conditions. Both *P. reptans* and *S. congestus* have a 60-day cold, moist stratification period recommended by the seed supplier, Prairie Moon Nursery (Winona, Minnesota). For *A. patens*, Prairie Moon recommends planting outside in the fall to allow for natural cold stratification. Natural stratification was not an option because the plants were intended for a controlled experiment, and waiting through a winter would delay the experiment. The optimal method for either *Eriophorum* species is unknown. We attempted germination after a 30-day cold, moist stratification period to determine if any of the species were opportunistic enough to germinate with less time in stratification.

To further reduce stratification duration, we treated seeds with one of two levels of gibberellic acid (GA₃). Gibberellic acid is a naturally occurring plant hormone used throughout the horticulture and agriculture industries to break seed dormancy and induce germination (Hartmann et al. 1990). Puttha et al. (2014) demonstrated the success of GA₃ treatment following stratification in their study examining germination of North American

native *Helianthus tuberosus* L. However, the use of GA₃ in native plant propagation is understudied.

1.2 Objectives

Our objectives in these studies were fourfold:

1. To determine if the selected species could germinate after a 30-day stratification
2. To determine an effective seed sterilization method capable of reducing fungal and bacterial growth without impacting seed viability
3. To confirm the media type most conducive for germination following stratification and to establish species-specific germination success following stratification
4. To determine if we could reduce stratification from 30 days to 15 days by using the plant-derived hormone GA₃ and to establish an optimal treatment concentration

1.3 Approach

Seeds from five native cold-hardy species were put through one of four surface sterilization treatments then stratified in a cold, moist environment for 30 days. Following stratification, they were examined for the presence of bacterial or fungal growth, or both. Seeds were then planted in potting soil and placed in a growth chamber to observe germination. The sterilization and stratification processes were repeated with a second group of seeds, which, after a 30-day stratification, were placed on moistened filter paper in Petri dishes and placed in the growth chamber. The Petri dishes allowed us to explore filter paper as germination media and continue observation of bacterial and fungal growth. In a separate experiment, the same five species were treated in one of two levels of GA₃, plus a control, to determine if the application of the hormone could reduce stratification time to 15 days.

2 Materials and Methods

We conducted two separate experiments to establish a standard operating procedure for seed sterilization and accelerated germination of the five plant species. We tested five species in a factorial 4×2 design to evaluate the effects of 4 types of sterilization and 2 growth media on germination success ($n = 24$ seeds per species per sterilization technique per growth media). In a separate experiment, we manipulated GA₃ concentration to determine if stratification time could be reduced ($n = 24$ seeds per treatment level). Across all experiments, only Type II lab water was used.

2.1 Seed Selection

The five cold-hardy species tested across all experiments were *Eriophorum vaginatum* L.; *Eriophorum virginicum* L.; *Polemonium reptans* L.; *Senecio congestus* DC.; and *Anemone patens* var. *multifida* Pritz., Linnaea.

These species were selected for their tolerance of colder environments, with three out of the five species being native to Alaska (Table 1). *E. vaginatum* seeds were obtained from Pase Seeds (North Collins, New York). All other species came from Prairie Moon Nursery (Winona, Minnesota).

Table 1. Species taxonomic information, native range and growth preferences.

Species	Common Name	Family ³	Class ³	Life Cycle ³	Soil Moisture	Native to These States ¹	Sun Exposure	USDA Zones	Recommended Stratification Method	Source
<i>Anemone patens</i> var. <i>multifida</i>	Pasque flower or prairie crocus	<i>Ranunculaceae</i>	Dicot	Perennial	Medium dry, dry	AK, CO, ID, IL, IA, KS, MI, MN, MT, NE, NM, ND, SD, TX, UT, WA, WI, WY	Full, partial	3 to 6	Plant outside in fall	Prairie Moon (2023a)
<i>Eriophorum vaginatum</i> L.	Tussock cottongrass	<i>Cyperaceae</i> Juss.	Monocot	Perennial	Wet	AK, CT, IN, MA, ME, MI, MN, NH, NJ, NY, PA, RI, VT, WI	Full	4	Unknown	Pase Seeds (2023)
<i>Eriophorum virginicum</i> L.	Tawny cottongrass	<i>Cyperaceae</i> Juss.	Monocot	Perennial	Wet	CT, DC, DE, GA, IA, IL, IN, KY, MA, MD, ME, MI, MN, NC, NH, NJ, NY, OH, PA, RI, SC, TN, VA, VT, WI, WV	Full, partial	3 to 9	Unknown	Prairie Moon (2023c)
<i>Polemonium reptans</i> L.	Jacob's ladder	<i>Polemoniaceae</i>	Dicot	Perennial	Medium wet, medium, medium dry	AL, AR, CT, DC, DE, GA, IA, IL, IN, KS, KY, MA, MD, MI, MN, MO, MS, NC, NH, NJ, NY, OH, OK, PA, RI, SD, TN, VA, VT, WI, WV	Full, partial, shade	3 to 8	60 days cold, moist stratification	Prairie Moon (2023d)
<i>Senecio congestus</i> DC.	Marsh fleabane	<i>Asteraceae</i>	Dicot	Annual/biennial	Wet, medium wet	AK, IA, MI, MN, ND, SD, WI	Full	3 to 5	60 days cold, moist stratification	Prairie Moon (2023e)

Note: Unless noted at the column header, the information was obtained from the seed source.

¹ USDA, NRCS (2023).

2.2 Sterilization Treatments

To identify an effective seed sterilization method, 24 seeds from each species underwent one of four surface-sterilizing treatments (Group 1 in Figure 1). The sterilization procedure was repeated a second time to provide enough seeds for the germination media treatments following stratification (Group 2 in Figure 1). The four treatments, detailed below, were 70% ethanol solution, 5% hydrogen peroxide solution, UVC light, or no sterilization (control). For stratification, all 24 seeds for a species in a treatment were placed between two pieces of filter paper (Q 8 Fisher Scientific, Pittsburgh, Pennsylvania) saturated in Type II lab water and placed in plastic 12.5 in.* diameter Petri dishes with covers and sealed with parafilm. Petri dishes were sterilized with 70% ethanol before use. Once containing seeds, dishes were stored at 4°C for 30 days in a mainly dark environment for stratification.

Surface sanitizing treatments:

a. Ethanol (EtOH) plus Type II lab water

To achieve an ethanol concentration of 70%, 95% ethanol was diluted with Type II lab water. Seeds were soaked in solution under constant agitation via a stir plate for 60 s, then moved to a Type II lab water rinse for 2–3 min.

b. Hydrogen peroxide (H₂O₂) plus Type II lab water

To achieve a concentration of 5%, 10% hydrogen peroxide was diluted with Type II lab water. Seeds were soaked in solution under constant agitation via a stir plate for 60 s, then moved to a Type II lab water rinse for 2–3 min.

c. UVC light

In a tray, seeds were exposed to a handheld UVC light for a 10 s interval with the light 0.5 in. above the seeds. The tray was shaken to expose

* For a full list of the spelled-out forms of the units of measure used in this document and their conversions, please refer to *US Government Publishing Office Style Manual*, 31st ed. (Washington, DC: US Government Publishing Office, 2016), 248–52 and 345–47, <https://www.govinfo.gov/content/pkg/GPO-STYLEMANUAL-2016/pdf/GPO-STYLEMANUAL-2016.pdf>.

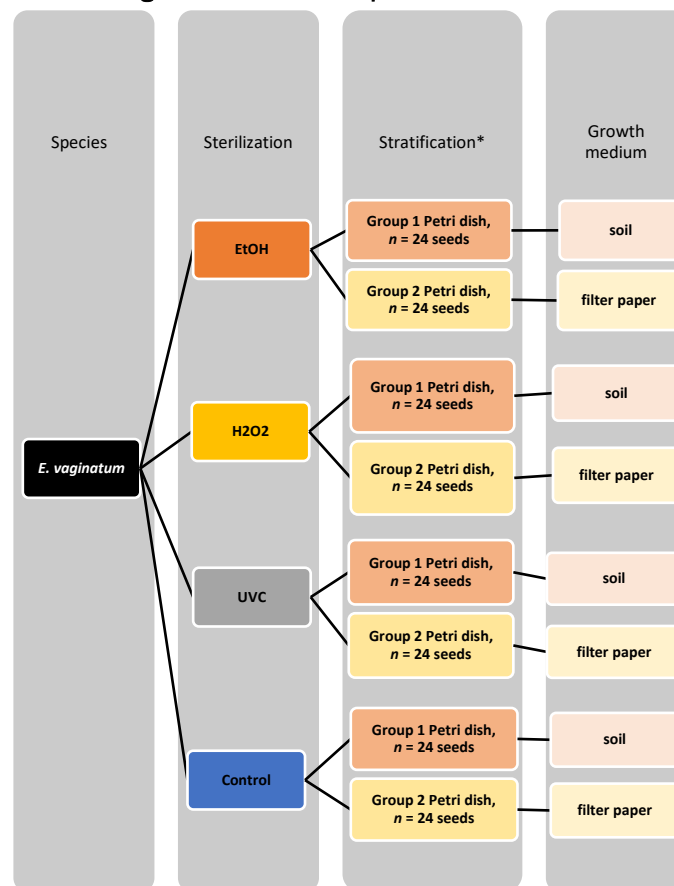
another side of the seed surface and exposed to a second 10 s interval of UVC light at a 0.5 in. distance.

d. Control

Seeds from each species were placed directly onto saturated filter paper within Petri dishes. Forceps were dipped in 70% ethanol between species to prevent cross contamination.

Following the 30-day stratification, the effectiveness of the sterilization treatment was recorded for individual seeds in Groups 1 and 2 (Table 2). Discoloration or fungal growth on the filter paper immediately surrounding a seed served as indication of contamination.

Figure 1. Experimental design for *Eriophorum vaginatum*, which was repeated for all five species. Color coding for growth medium is repeated in Table 2.



*Stratification was not a treatment. The separation into two groups is meant to illustrate stratification taking place separately for the two groups of seeds. One group would be planted in soil and the second group placed on filter paper in petri dishes.

2.3 Growth Media Germination

After the 30-day stratification period, seeds were transferred to either sterilized soil or moistened filter paper to be germinated. For the soil media group, standard potting soil was pressed through a 2.00 mm (No. 10) sieve to create a substrate of a finer texture and to improve seed-to-soil contact. The sieved soil was placed in a drying oven for 30 min at 120°C, allowed to cool, and mixed with Type II lab water. Water was incorporated to the point where the soil was uniformly moist but not saturated. Soil was placed in six-cell plug tray inserts, and four seeds of one species were sown in a cell, to fill six cells per species. All five species of a treatment group were placed in a water catchment tray. Species were randomly assigned cells within a tray insert to account for environmental differences in the growth chamber. Despite growing in a shared space, each seed was considered a replicate for a total of 24 replicates per species per treatment. Seeds were transferred from the filter paper via forceps and were gently pressed onto the soil surface to create good contact. Seeds were watered, covered with a clear dome, and transferred to the growth chamber (Model PGC-105, Percival Scientific, Perry, Iowa). The chamber was programmed for 24 h of light with a temp of 18°C and relative humidity of 60%, as reported in Müller et al. (2011).

For germination on filter paper, eight seeds from each species were evenly dispersed onto two new pieces of lab-water-saturated filter paper and placed in 70% ethanol sterilized plastic 12.5 in. diameter Petri dishes with covers. Three dishes in total were prepared with eight seeds each. We considered a single seed as a replicate. The dishes were placed in trays and transferred to the growth chamber where the environmental conditions mirrored those of the soil media germination treatment described above. For this part of the experiment, we both monitored germination and continued seed sterilization treatment effect. Discoloration or fungal growth on the filter paper immediately surrounding a seed served as indication for contamination. Contamination was recorded immediately following stratification and once a week for a 3-week period following the transition of seeds to the growth chamber. The percentage of contaminated seeds was reported for each individual species in a sterilization treatment.

Successful germination for the purpose of filter-paper germination is defined as the complete emergence of one or more cotyledons from the seed coat; and for soil media, it is the presence of one or more cotyledons

above the soil line. Seedling emergence was observed and recorded three times a week over the 21 days following the transitioning of seeds to the growth chamber.

During the germination phase, seeds sown in soil were watered according to their preferred level of wetness, and those on filter paper were kept consistently moist.

2.4 Reducing Stratification Time with Gibberellic Acid

We tested if a treatment with GA₃ could overcome a truncated stratification period. Following results from the previous experiment, we used 70% ethanol to sterilize 72 seeds from each species. Seeds were removed from stratification after 15 days and put through one of three levels of a gibberellic acid treatment, 0 mg/L, 500 mg/L, or 1000 mg/L ($n = 24$ per species per treatment). For each treatment, the GA₃ was first dissolved in 30 mL of 95% ethanol under agitation on a stir plate for 5 min. It was then mixed with 1 L of Type II lab water and divided into five beakers, one for each species, resulting in 200 mL of solution in each. Seeds were added and allowed to soak for 24 h. They were then filtered out, rinsed, and directly sown in six-cell plug inserts placed in trays. Four seeds of one species were planted to a cell with the five species assigned randomly to placement within an insert. Inserts within a treatment were grouped in a water catchment tray. The potting soil was prepared using the method outlined previously, and trays were placed in the growth chamber under the same environmental conditions. Germination observation and definition for the purpose of this experiment was as described in the previous method.

3 Results

3.1 Sterilization Treatment Results

Immediately following the 30-day cold, moist stratification, those seeds treated in 70% ethanol showed no visible contamination across all species. All *A. patens* seeds had discoloration on the filter paper for the other treatment groups. *E. virginicum* seeds in control Group 1 were all contaminated. Of the other species in control Group 1, a select number of seeds also showed signs of contamination (Table 2).

Table 2. Count of seeds with contamination following stratification for Group 1 (soil growth medium) and Group 2 (filter paper growth medium) and new contamination after the 21-day germination period in the growth chamber for Group 2. Shading matches Figure 1.

Treatment and Species	Count of Seed Contamination Immediately Following Stratification		Count following 21-Day Germination Period
	Group 1*	Group 2	Group 2
Control			
<i>Anemone patens</i>	24 (100%)	24 (100%)	N/A
<i>Eriophorum vaginatum</i>	1	0	8
<i>Eriophorum virginicum</i>	24 (100%)	0	8
<i>Polemonium reptans</i>	2	0	13
<i>Senecio congestus</i>	1	0	2
UVC			
<i>Anemone patens</i>	24 (100%)	24 (100%)	N/A
<i>Eriophorum vaginatum</i>	0	0	15
<i>Eriophorum virginicum</i>	0	0	7
<i>Polemonium reptans</i>	0	0	22
<i>Senecio congestus</i>	0	0	5
H₂O₂			
<i>Anemone patens</i>	24 (100%)	24 (100%)	N/A
<i>Eriophorum vaginatum</i>	0	0	7
<i>Eriophorum virginicum</i>	0	0	3
<i>Polemonium reptans</i>	0	0	12
<i>Senecio congestus</i>	0	0	2
EtOH			
<i>Anemone patens</i>	0	0	11
<i>Eriophorum vaginatum</i>	0	0	4
<i>Eriophorum virginicum</i>	0	0	1
<i>Polemonium reptans</i>	0	0	0
<i>Senecio congestus</i>	0	0	3

* Seeds from Group 1 were used for the soil germination experiment and could not be evaluated for contamination following the 21-day germination period.

The seeds in Group 2 were used in the filter-paper germination trial. Once the seeds were transferred into their new dishes and placed in the warmer germination environment, new occurrences of fungal and bacterial growth were recorded. With the exception of *S. congestus*, the treatment of 70% ethanol was most effective in reducing contamination across the different species for the total observation time (Figure 2). The percent contaminated seed following EtOH treatment was 16% when averaging across all species (Table 3).

Figure 2. Number of Group 2 seeds contaminated within each treatment, grouped by species. Figure shows contamination over the total observation time.

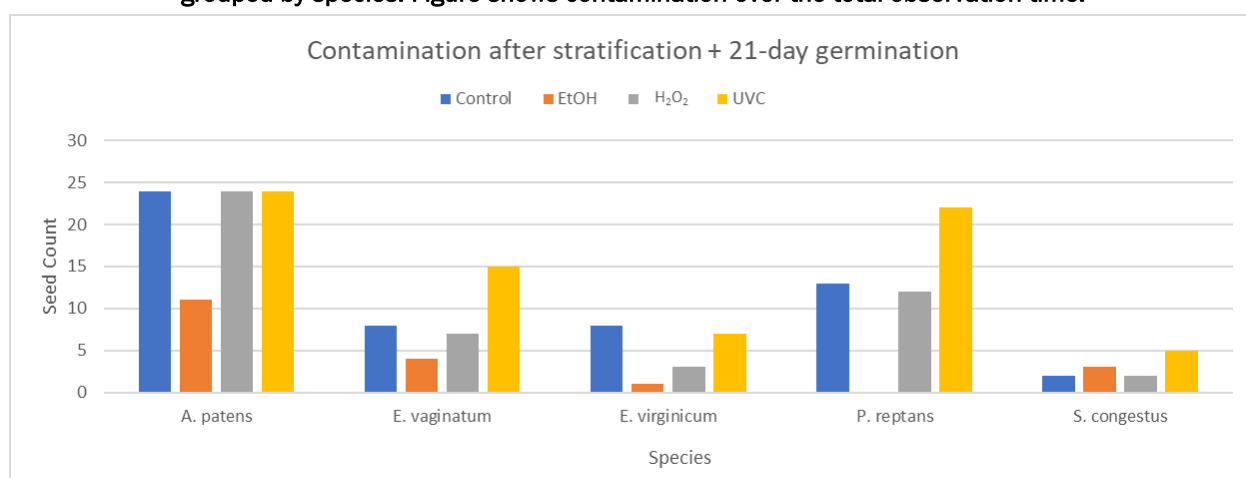


Table 3. Percentage of contaminated seed per species and sterilization treatment and the average percent contamination for all species in the treatment (Group 2, after stratification and 21-day germination time).

Treatment	<i>A. patens</i>	%	<i>E. vaginatum</i>	%	<i>E. virginicum</i>	%	<i>P. reptans</i>	%	<i>S. congestus</i>	%	Avg %
Control	24	100%	8	33%	8	33%	13	54%	2	8%	46%
EtOH	11	46%	4	17%	1	4%	0	0%	3	13%	16%
H ₂ O ₂	24	100%	7	29%	3	13%	12	50%	2	8%	40%
UVC	24	100%	15	63%	7	29%	22	92%	5	21%	61%

3.2 Growth Media Germination Results

For both soil and filter-paper media, *E. vaginatum* was the only species to germinate within the 21-day period. The control group had the highest germination when using soil for the germination media whereas the seeds treated in 70% ethanol had the highest germination on filter paper (Table 4).

Table 4. The number of *E. vaginatum* seeds germinated from each sterilization treatment and media type over a 21-day period.

Treatment	Germination Media			
	Soil		Filter Paper	
	Count	%	Count	%
Control	5	21	2	8
H ₂ O ₂	1	4	1	4
UVC	4	17	0	0
EtOH	0	0	4	17
Total	10	10	7	7

3.3 Gibberellic Acid Results

The GA₃ treatments of 500 ppm (parts per million) and 1000 ppm had the greatest impact on *P. reptans*, with 29% of treated seeds germinating at the 500 ppm level and 42% germination at 1000 ppm. In the control group for this species, germination was 0% (Table 5). Although 13% of the *E. vaginatum* seeds treated at 500 ppm germinated, germination was considerably low and not sufficiently higher than the control group. Neither GA₃ treatment stimulated germination in the other species tested.

Table 5. Number of seeds germinated over a 21-day period by species and gibberellic acid treatment level.

21- Day Germination	<i>A. patens</i>		<i>E. vaginatum</i>		<i>E. virginicum</i>		<i>P. reptans</i>		<i>S. congestus</i>	
	#	%	#	%	#	%	#	%	#	%
0 ppm	0	0	2	8	0	0	0	0	0	0
500 ppm	0	0	3	13	0	0	7	29	0	0
1000 ppm	0	0	1	4	0	0	10	42	0	0
Total Count	0	—	6	—	0	—	17	—	0	—

4 Conclusion

There are several things to consider from these results. The seeds monitored for the full stratification and 21-day germination period (Group 2), demonstrated 70% ethanol as an effective seed surface sterilant. The 70% EtOH provided an average of 84% successful sterilization across all five species tested. Because the viability of seeds was not tested beforehand, the low or unsuccessful germination across treatments could be the result of low viability rather than sterilization effects.

The results for media type efficacy to germinate *E. vaginatum* demonstrate there is no difference between potting soil and filter paper. These results are beneficial to a research program as planting directly in soil eliminates the step of transplanting, which would be needed for seeds started on filter paper. Use of a soil medium reduces not only staff effort but also production time since plants need time to acclimate following transplanting. Transplanting also introduces an opportunity for pathogen introduction or plant injury. Again, this potting soil was sterilized to limit microorganism activity as soil containing pathogenic fungi and bacteria can cause seed rot or seedling disease (Hartmann et al. 1990). Had the soil been untreated, issues relating to pathogen activity could have arisen due to the soil itself, rendering comparison to the filter paper unclear.

The addition of GA₃ showed effect at both the 500 ppm and 1000 ppm levels for two of five species, *P. reptans* and *E. vaginatum*. With these results, we can now germinate *P. reptans* in 36 days (15 day stratification, plus 21 germination) instead of 81 (60 day stratification, plus 21 day germination). However, since the stratification time of *E. vaginatum* is unknown and seeds germinated in the control group, it is unclear if GA₃ was the cause of germination. Our approach to apply GA₃ following stratification is novel for these species; more often GA₃ is applied before stratification.

The work presented here could be improved if the following are taken into consideration. With UVC light, it was challenging to exposing the entire seed surface to the treatment. A better method to expose the entire seed surface to the UVC, such as placing seeds in a beaker with a stir bar during exposure, may lead to different results. The filter paper itself was not sterilized before use and cannot be fully ruled out as a source of contamination. All *E. virginicum* seeds in sterilization control (no sterilization) Group 1 and at least one seed from the remaining three species were contaminated.

But none of these four species exhibited contamination in sterilization control Group 2. It is possible that the filter paper was the source of contamination for Group 1. In the filter-paper germination, the majority of *A. patens* seeds showed contamination by the end of the germination period. To reduce risk of contamination in the future, the filter paper can be autoclaved before use. Finally, testing seed viability before a germination study will eliminate questions around the effectiveness of a stratification period and hormone treatment. For the species with unknown stratification times, *E. vaginatum* and *E. virginicum*, germination trials for stratification would create a baseline against which the methods developed here of accelerated germination could be tested.

The methods developed here have since been used to propagate another Arctic species, *Caltha palustris* L. (*Ranunculaceae*), commonly known as marsh marigold. The seeds were surface sterilized using 70% EtOH and planted in sterile potting soil using the methods described in Sections 2.2 and 2.3. Before planting, seeds were placed in cold, moist stratification for 15 days, then put through one of three levels of a GA₃ described in Section 2.4. At the 1000 ppm level, we saw 36% germination, at 500 ppm 27% germination, and at 0 ppm 5% germination. *C. palustris* has a recommended 60-day cold, moist stratification (Prairie Moon Nursery 2023, “*Caltha palustris*.”). Therefore, using these methods saved 45 days of production time and yielded healthy plants to sample.

The need for accelerated propagation of Arctic plants is undeniable. As climate change thaws permafrost, current understanding of the Arctic ecosystem is becoming obsolete, with implications for military and civil infrastructure. The ability to reproduce Arctic vegetation enables research to identify potential performance issues, particularly concerning mobility. Additionally, thawing permafrost affects carbon storage in the Arctic. Quick and reliable propagation methods are necessary to populate computational models regarding changes in carbon sequestration. The work described here reduces the time necessary to solve problems and find answers in support of the Army mission.

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14. ABSTRACT We conducted growth chamber experiments to overcome challenges of native seed germination relating to disease and germination time. We selected five northern species, <i>Eriophorum vaginatum</i> , <i>E. virginicum</i> , <i>Anemone patens</i> var. <i>multifida</i> , <i>Polemonium reptans</i> , and <i>Senecio congestus</i> , for their native ranges and commercial-nursery availability. Recommended stratification time for each species was either unknown or a minimum of 60 days. Seeds were sterilized with 70% ethanol, 10% hydrogen peroxide, or UVC light to identify which method most effectively prevented pathogen infection. To determine if stratification time could be reduced, seeds underwent a 30-day cold, moist stratification. We tested which growth medium was most conducive to germination of the sterilized, stratified seeds: filter paper or sterilized potting soil. In a separate experiment, we tested if three different levels of gibberellic acid (GA ₃ ; 0, 500, and 1000 ppm) could reduce stratification time to 15 days. The 70% ethanol was effective in a seed surface sterilization; an average of 84% of all seeds for all species treated showed no contamination. Germination following a 30-day cold, moist stratification was unsuccessful for most species tested in both growth media. Here, 1000 ppm GA ₃ with a 15-day cold, moist stratification showed considerable success with <i>P. reptans</i> .					
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