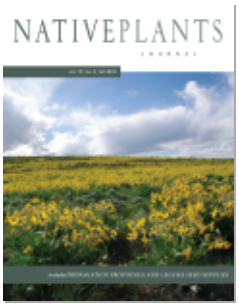
Germination ecology of native plant species for use in restoration and the urban landscape in Nova Scotia, Canada



Amy Heim, Ayako Nagase, Jeremy Lundholm, Carly Wrathall

Native Plants Journal, Volume 19, Number 3, Fall 2018, pp. 201-215
(Article)

Published by University of Wisconsin Press

Germination ecology of native plant species for use in restoration and the urban landscape in Nova Scotia, Canada

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ABSTRACT

In Nova Scotia, Canada, demand is high for native species for ecological restoration and use in the urban environment. The region has few native plant suppliers, however, which forces groups to seek resources outside their region or to grow their own vegetation. Collecting, storing, and germinating seeds facilitate access to large quantities of viable seeds for use in projects. We conducted 4 germination trials on 21 species native to Atlantic Canada. We examined germination response to various treatments including storage method (freshwater, dry, seeds, whole berries) and temperature (4 °C [39.2 °F]), –20 °C [–4 °F]), as well as germination irrigation treatments (saltwater, freshwater). Overall, we found that species preferred treatments that reflected their natural environment. Therefore, for Atlantic Canada, we recommend that species found in wet habitats should be stored in freshwater and species found in dry habitats should be stored dry, both conditions at 4 °C (39.2 °F).

Heim A, Nagase A, Lundholm J, Wrathall C. 2018. Germination ecology of native plant species for use in restoration and the urban landscape in Nova Scotia, Canada. Native Plants Journal 19(3):201–215.

KEY WORDS

seed storage, saltwater, propagation, temperature

NOMENCLATURE

Kartesz and Meacham (1999)

CONVERSIONS
(°C × 1.8) + 32 = °F
mm × 0.04 = in
km × 0.62 = mi

Interest in using native plant species is increasing for landscaping, private gardens, and ecological restoration projects. Many landscape architects now consider plant conservation and climate adaptations when choosing prospective vegetation (Brzuszek and others 2007), and ecological restoration projects generally require native species when planting is suggested. The Atlantic provinces of Canada (New Brunswick, Newfoundland and Labrador, Nova Scotia, and Prince Edward Island) are home to more than 1500 species of native vascular plants (ACCDC 2017). The region has few native plant suppliers, however, which forces practitioners to seek resources outside their region or to grow their own plants. In Nova Scotia, demand is for native species for salt marsh restoration and use in the urban environment (for example, green roofs). The types of plant species needed for salt marsh restoration include those commonly found across a range of tidal regimes from low salt marsh to brackish systems (Porter and others 2015) including dominant grasses (*Spartina* spp. [Poaceae]). For use in the urban environment, roadside and coastal plants (for example, *Festuca rubra* L. [Poaceae]) are a viable option as they can survive harsh environments exposed to rapid drainage, strong winds, and salt spray (Nellis 1994). Coastal plants have already been successfully established on green roofs in the province (MacIvor and Lundholm 2011; Heim and others 2016). To better promote the use of native plants in Atlantic Canada we must first understand their germination ecology.

Collecting, storing, and germinating seeds facilitate access to large quantities of viable seeds for use in projects related to ecological restoration and green infrastructure. How these seeds are stored has the potential to affect germination rates, and different plant species require specific storage and preparation methods. For example, wet or dry stratification of seeds has been extensively studied in *Spartina* spp., showing that wet stratification enhances germination of these species (Callaway and Josselyn 1992; Bruno 2000; Chung and others 2004). As *Spartina* spp. grow primarily in salt marshes, this storage method reflects what seeds would be exposed to in the natural environment. Applying this reasoning to other regional native species that have not been studied from the perspective of propagation will provide us with a stronger understanding of germination requirements for individual species, allowing growers to increase the quantity of species used in restoration and green infrastructure.

To understand the germination ecology of species native to Nova Scotia, we have compiled 4 germination trials. These trials provide information on seed storage and treatment methods for 21 species native to Atlantic Canada. Some of the species examined have been extensively studied in other geographic locations (for example, *Spartina* spp.) (Callaway and Josselyn 1992; Bruno 2000; Chung and others 2004). Given that plant traits can be geographically variable (Mobberley 1956; Fang and others 2004), however, we wanted to specifically test seeds gathered from Nova Scotia.

METHODS

Between 2005 and 2017, 4 germination trials were conducted on 21 species native to Nova Scotia. These species were selected for their potential to survive on green roofs (species found on coastal barrens, roadsides, dunes) or because they are used in salt marsh restoration (species found on salt marshes). The majority of seeds were collected around Halifax, Nova Scotia, which has an average yearly temperature of 7.7 °C and an average yearly precipitation of 1468.1 mm (Government Canada 2018). All germination trials were conducted in growth chambers at Saint Mary's University, located in Halifax (44.63222 N, 63.58139 W) (Figure 1B, 1C). For each species, all berries and (or) seeds were collected by hand from the same grouping of plants and placed in one collection container. Germination trials examined germination rates for different pre-storage, temperature, and irrigation treatments (Table 1). Each trial ended after 2 wk with no germination.

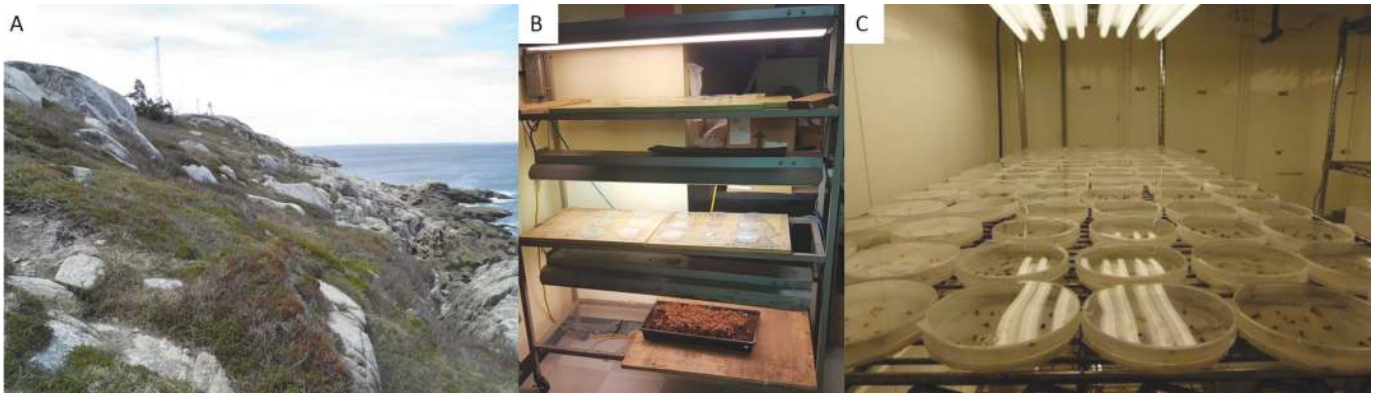

Figure 1. One of the coastal barren seed collection sites (Chubucto Head) (A), the growth chamber used in Trials 1 and 4 (B), and the growth chamber used in Trials 2 and 3 (C). Photo by Amy Heim

TABLE 1

Overview of the 4 germination trials.

Trial	Plant species	Month Year collected	Pre-storage treatment	Storage temperature	Storage duration	Irrigation treatment	Germination duration
1	*Empetrum nigrum* *Empetrum eamesii* *Vaccinium angustifolium* *Gaylussacia dumosa* *Vaccinium macrocarpon*	August 2004 September 2004 October 2004	Seeds Berries	4 °C –20 °C	6 mo	Freshwater	2 mo
2	*Juncus gerardii* *Festuca rubra* *Schoenoplectus maritimus* *Spartina patens* *Spartina pectinata* *Anaphalis margaritacea* *Atriplex glabriusculata* *Lathyrus japonicus* *Limonium carolinianum* *Oenothera biennis* *Plantago maritima* *Solidago sempervirens* *Spartina alterniflora* *Symphyotrichum novi-belgii*	July 2013 August 2013 September 2013 October 2013	Freshwater dry	4 °C	2.5 mo	Freshwater	2.5 mo
3	*Spartina alterniflora* *Spartina patens* *Spartina pectinata*	October 2014	Freshwater	4 °C –20 °C	2.5 mo	Freshwater Saltwater	34 d
4	*Calamagrostis pickeringii* *Hudsonia ericoides* *Limonium carolinianum* *Plantago maritima*	August 2015 October 2015	Freshwater dry	4 °C	2 y	Freshwater Saltwater	2.5 mo

Trial 1: Storage at 4 °C vs. –20 °C and Seeds vs. Berries

In 2004, berries from *Empetrum nigrum* L. (black crowberry [Ericaceae]), *Empetrum eamesii* Fernald & Wiegand (purple crowberry [Ericaceae; some sources place *Empetrum* in Empetraceae]), *Vaccinium angustifolium* Aiton (lowbush blueberry [Ericaceae]) (Figure 2), *Gaylussacia dumosa* Fern. (dwarf huckleberry [Ericaceae]), and *Vaccinium macrocarpon* Aiton (cranberry [Ericaceae]) (Figure 3) were collected from Chubucto Head, a coastal barren site located 16 km from Halifax, Nova Scotia (see Table 1; Figure 1A). Although it is difficult to differentiate individual plants in these clonal shrubs, berries from approximately 25 to 50 plants were collected for each species.

Immediately after collection, berries were separated into different storage treatments: dry storage at 4 °C for individual seeds (seeds removed from fruit with tweezers) or whole berries, and dry storage at –20 °C for individual seeds or whole berries (*V. macrocarpon* and *G. dumosa* were stored at only 4 °C). Germination trials began on 11 May 2005 and ended on 16 August 2005. Seeds and (or) berries were germinated at room temperature (17–20 °C) under a growth light, in plastic plant cells (1 per cell) that contained soil that was kept moist throughout the entire trial. The number of seeds and (or) berries for each replicate was chosen based on the average number

Figure 2. Vaccinium angustifolium (Green roof, Saint Mary's University). Photo by Amy Heim

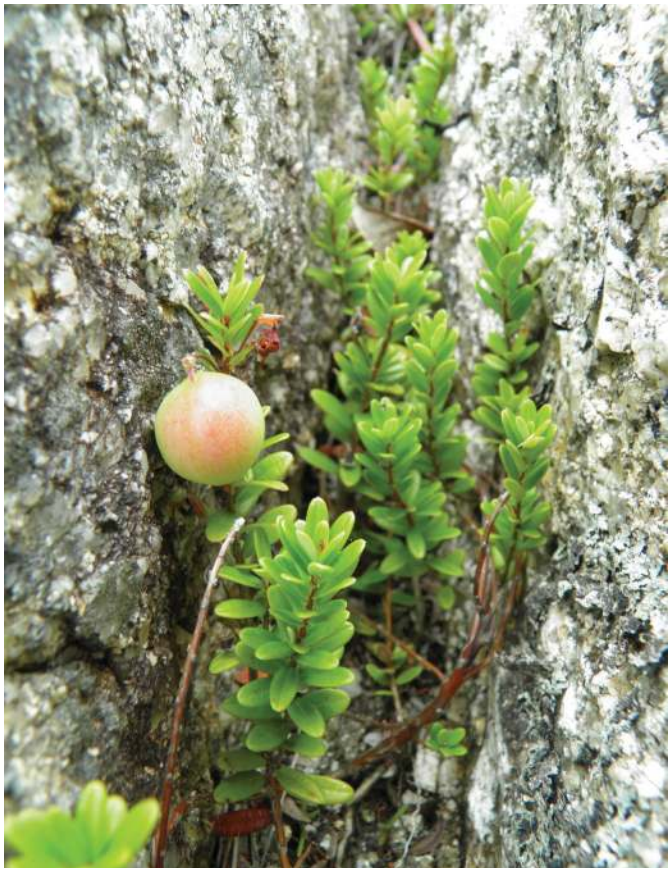

Figure 3. Vaccinium macrocarpon (Duncan's Cove Nature Reserve, Nova Scotia). Photo by Amy Heim

of seeds found in a typical berry for that species (see Appendix Table A.2).

Trial 2: Wet vs. Dry Storage

In 2013, seeds were collected from the Chubucto head coastal barren (*Oenothera biennis* L. [common evening primrose; Onagraceae], *Plantago maritima* L. [goose tongue; Plantaginaceae]) (Figure 4); the green roof at Saint Mary's University (located in Halifax) (*Anaphalis margaritacea* (L.) Benth. [western pearly everlasting; Asteraceae], *Festuca rubra* L. [red fescue; Poaceae], *Symphyotrichum novi-belgii* L. [New York aster; Asteraceae]); and the Conrad's Beach salt marsh (17 km from Halifax) (*Atriplex glabriusculata* Edmondston [Scotland orache; Chenopodiaceae], *Juncus gerardii* Loisel. [saltmeadow rush; Juncaceae], *Lathyrus japonicus* Willd. [beach pea; Fabaceae] (Figure 5), *Limonium carolinianum* (Walter) Britton [lavender thrift; Plumbaginaceae], *Schoenoplectus maritimus* (L.) Nels. [cosmopolitan bulrush; Cyperaceae], *Solidago sempervirens* L. [seaside goldenrod; Asteraceae], *Spartina alterniflora* Loisel. [smooth cordgrass; Poaceae], *Spartina patens* (Aiton) Muhl. [saltmeadow cordgrass], *Spartina pectinata* Bosc ex Link [prairie cordgrass]) (see Table 1). For the forbs, seeds were collected from 20 to 40 individual plants per species. For grasses, seeds were collected from 20 to 100 individuals per species.

Figure 4. Plantago maritima (Crystal Crescent Provincial Park, Nova Scotia). Photo by Jeremy Lundholm

Figure 5. Lathyrus japonicus (Crystal Crescent Provincial Park, Nova Scotia). Photo by Jeremy Lundholm

Once collected, seeds were stored dry at room temperature (20 °C) until the storage treatment began on 18 October 2013. This trial included 2 storage treatments: half the seeds for each species were stored dry at 4 °C in a moisture-free container. The second half of the seeds were placed in a sealable bag, moistened in tap water, and stored at 4 °C. From January 2014 until May 2014, seeds were germinated at room temperature (20 °C) under a full-spectrum fluorescent growth light in covered Petri plates containing moistened filter paper (see Figure 1C, Figure 6). During the trials, treatments were continually moistened with deionized water (~2 ml with each irrigation) to ensure water availability for the seeds.

Trial 3: Storage at 4 °C vs. –20°C and Freshwater vs. Saltwater Irrigation

In October 2014, seeds from *S. alterniflora, S. patens, and S. pectinata* were collected from 4 salt marsh sites: Conrad's

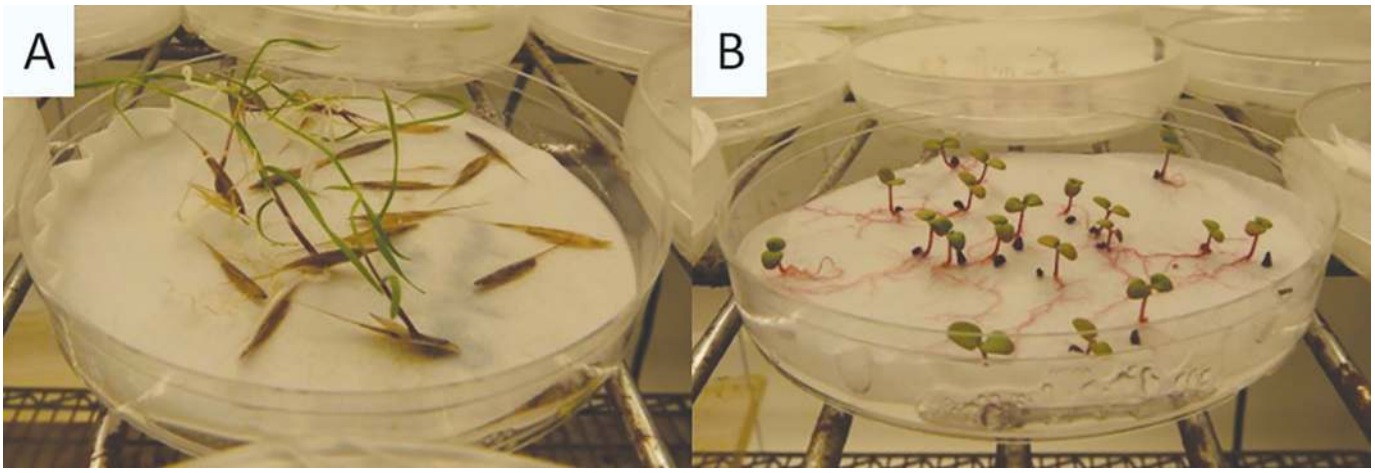

Figure 6. Spartina pectinata (A) and *Oenothera biennis* (B) germinating in the wet treatment during the 2013 trial. Photo by Amy Heim

Beach and Rainbow Haven (13 km from Halifax), which are located on the Atlantic Coast of Nova Scotia, and the Windsor (57 km from Halifax) and Cogmagun salt marsh (60 km from Halifax), which are located on the Bay of Fundy Coast of Nova Scotia (see Table 1). Compared to Halifax, the Bay of Fundy region has an average yearly temperature of 7.4 °C and an average yearly precipitation of 1309.6 mm. Seeds were collected from 20 to 40 individuals per species. Immediately after collection, seeds were washed in tap water and sprayed with a 5% concentration of Safer's Defender Garden fungicide to reduce mold (Fang and others 2004; Li and others 2010). Seeds were stored damp (~10 ml of water) at 4 °C for 4 d and then stored damp at 4 °C for 2.5 mo. From January to February 2015, seeds were germinated at room temperature (20 °C) under a full-spectrum fluorescent growth light in covered Petri plates containing filter paper (see Figure 1C). During germination, half the seeds were irrigated with freshwater (~2 ml with each irrigation) and the other half were irrigated with saltwater (~2 ml with each irrigation, salt and tap water [1% NaCl]).

Trial 4: Freshwater vs. Dry Storage and Saltwater vs. Freshwater Irrigation

In 2015 seeds from *P. maritima, Calamagrostis pickeringii* A. Gray (Pickering's reedgrass [Poaceae]), and *Hudsonia ericoides* L. (pine barren goldenheather [Cistaceae]) (Figure 7) were collected from Chubucto Head, and seeds from *L. carolinianum* were collected from Conrad's Beach (see Table 1). Seeds were collected from 15 to 20 individuals for *Hudsonia* and 30 to 50 individuals for the other species. Half of these seeds were stored dry, and half were stored wet (see Trial 2). From September to October 2017, seeds were germinated at room temperature (20 °C) under a full-spectrum fluorescent growth light in covered Petri plates containing filter paper (see Figure 1B). During germination, *P. maritima, C. pickeringii*, and *H. ericoides* seeds were irrigated with freshwater (~2 ml with each irrigation). For *P. maritima*, half the seeds were also irrigated once at the beginning of the germination period with saltwater (5 ml [0.17 fl oz]) (3% Reef Crystals Reef Salt). Saltwater was added only once, as seeds were not rinsed between irrigation and we wanted to avoid a build-up of salt.

Figure 7. Hudsonia ericoides (Duncan's Cove Nature Reserve, Nova Scotia). Photo by Jeremy Lundholm

Statistical Analysis

A one-way ANOVA was used to compare treatments using 2 storage methods: moist vs. dry stratification. A two-way ANOVA and Tukey post hoc test were used to examine

treatments using more than 2 methods: dry stratification at 4 °C vs. –20 °C for whole berry vs. seeds; stratification with freshwater vs. saltwater during storage at 4 °C and –20 °C; and freshwater vs. saltwater irrigation during germination with seeds that were stored wet and dry. For each data set, a Shapiro-Wilk test was used to test for normality and transformed if data were not normally distributed (see Appendix Tables) (R Project for Statistical Computing version 3.1.1; Warton and Hui 2011).

TABLE 2

Average germination rate (%) ± SE and number of seeds per replicate (x/x) for species in Trial 1.

Storage temperature	4 °C	20 °C	4 °C	20 °C
Storage treatment	Seeds	Seeds	Berry	Berry
Empetrum nigrum (x/8)	25.7±5.8	0.1±.06	0	0
Vaccinium angustifolium (x/41)	14.8±2.2	0	0	0
Empetrum eamesii (x/8)	0	14.6±4.1	0	0
Gaylussacia dumosa (x/10)	0	0	0	0
Vaccinium macrocarpon (x/16)	65.6±4.8	NA	2.8±2.8	NA

Notes: Replicates for *Gaylussacia dumosa* treatments were $n = 9$, and replicates for all other treatments were $n = 18$. SE, standard error.

RESULTS

Trial 1: Storage at 4 °C vs. –20 °C and Seeds vs. Berries

No germination was observed for *G. dumosa, V. angustifolium* germinated only in the seeds at 4 °C treatment, and *E. rubrum* germinated only in the seeds at –20 °C treatment. *Empetrum nigrum* germinated only when planted as seeds, with the highest germination observed for the seeds at 4 °C treatment. For *V. macrocarpon*, the highest germination occurred when planted as seeds (Table 2). Although details were not recorded, authors also observed that the seeds of *V. angustifolium* germinated en masse 2 mo into the experiment, whereas the seedlings of the *V. macrocarpon* and the *Empetrum* spp. germinated within 2 wk.

Trial 2: Wet vs. Dry Storage

No germination was observed for *C. pickeringii* or *J. gerardii*, therefore, these species were excluded from the statistical analysis. The cold, moist stratification treatment significantly increased the germination rate for 8 species (*A. glabriusculata, O. biennis, P. maritima, S. sempervivum, S. alterniflora, S. patens, S. pectinata,* and *S. novi-belgii*) and decreased the number of days until germination for 9 species (*A. glabriusculata, F. rubra, L. carolinianum, S. sempervivum, S. alterniflora, S. patens, S. pectinata,* and *S. novi-belgii*). Only 1 species, *O. biennis,* germinated significantly earlier in the dry treatment (Table 3).

TABLE 3

Average germination rate and average number of days until germination ± SE for species in Trial 2.

Species	Germination rate (%)		Average days until germination	
Trial 2	Wet (x/20)	Dry (x/20)	Wet	Dry
Anaphalis margaritacea	13.3±6.0	3.3±3.3	12.2±0.4	14.0±NA
Atriplex glabrisculata	96.7±3.3*	70.0±7.6*	4.1±0.2*	27.6±3.7*
Festuca rubra	58.3 ±3.3	53.3±1.7	6.4±0.3*	10.0±0.3*
Lathyrus japonicus	0.0±0.0	1.7±1.7	NA	6 ±NA
Limonium carolinianum	65.0±7.6	50.0±2.9	6.4±0.6*	21.6±0.7*
Oenothera biennis	95.0±2.9*	15.0±2.9*	3.3±1.0*	9.5±3.6*
Plantago maritima	95.0±2.9*	13.3±1.7*	3.4±0.1	10.9±4.9
Schoenoplectus maritimus	10.0±7.6	10.0±5.8	7.9±2.4	15.9±5.5
Solidago sempervirens	85.0±7.6	36.7±7.3*	4.3±0.1*	10.5±1.1*
Spartina alterniflora	35.0±5.8*	18.3±1.7*	19.2±3.0*	31.5±1.7*
Spartina patens	28.3±3.3*	6.7±1.7*	6.3±0.6*	47.0±7.5*
Spartina pectinata	40.0±8.7*	6.7±1.7*	6.3±0.4*	44.2±5.1*
Symphyotrichum novi-belgii	51.7±9.3	38.3±9.3	4.9±0.3*	19.0±3.3*

Notes: Each species was stored at 4 °C and had a freshwater and a dry storage treatment. Trial 2 had 3 replicates with 20 seeds per replicate. For each species and category, an * indicates significant difference between treatments ($P < 0.05$). Only 1 replicate of *Lathyrus japonicus* germinated, and only 1 replicate of *Anaphalis margaritacea* germinated in the dry treatment, therefore, the average could not be calculated for these species. SE, standard error.

Trial 3: Storage at 4 °C vs. –20 °C and Freshwater vs. Saltwater Irrigation

Compared to all other treatments, both *S. pectinata and S. patens* had a significantly higher germination rate in the freshwater at 4 °C treatment. For *S. alterniflora*, no seeds germinated in the freshwater at –20 °C treatment, and only 1 replicate germinated in the saltwater at –20 °C treatment. *Spartina alterniflora* had a significantly higher germination rate when stored at 4 °C, with no significant difference observed between freshwater and saltwater treatments. For all 3 species, the freshwater at 4 °C treatment resulted in the quickest germination time (Figure 8).

Trial 4: Freshwater vs. Dry and Saltwater vs. Freshwater

For Trial 4, the dry storage/freshwater treatment of *P. maritima* had a significantly higher germination rate than all other treatments. Additionally, *P. maritima* germinated significantly faster in the wet storage/freshwater treatment than in the dry storage/saltwater treatment. *Limonium carolinianum* had a significantly greater germination rate in the dry treatment; however, it had a significantly shorter delay in germination in the wet treatment. No significant difference was observed for *H. ericoides*; 2 of the 3 wet treatments for *H. ericoides* did not germinate (Table 4).

DISCUSSION

Out of the 21 species examined, all but 6 had a treatment that resulted in a significant increase in germination and (or) a significant decrease in the number of days until germination. Out of the 6 species that did not vary significantly between treatments (*C. pickeringii, G. dumosa*, *J. gerardii*, *A. margaritacea*, *L. japonicus,* and *S. maritimus*), all had zero to low germination (between 0% and > 13.5%). For those species with a preferred treatment, the methods used often reflected each species' natural environment. This trend is also reflected in the literature, with species from wet habitats responding positively to wet stratification (Fenner and Thompson 2005; Liu and others 2010; Baskin and Baskin 2014) and species from cold,

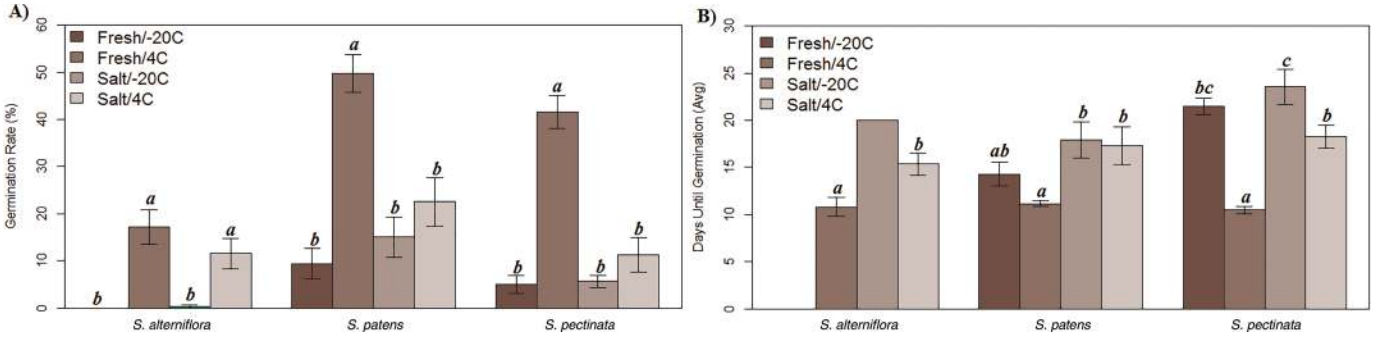

Figure 8. Average germination rate (A) and average number of days until germination (B) for *Spartina alterniflora*, *S. patens*, and *S. pectinata* when stored in freshwater (fresh) vs. saltwater (salt) at 4 °C vs. –20 °C. *Spartina alterniflora* in the freshwater at –20 °C treatment did not germinate, and only 1 replicate germinated in the saltwater at –20 °C treatment. For each species, those bars that share a letter are not significantly different. Error bar indicates standard error.

TABLE 4

Trial 4: Freshwater vs. dry storage for Plantago maritima, Hudsonia ericoides, *and* Limonium carolinianum, *and the use of saltwater vs. freshwater irrigation for* Plantago maritima.

Species	Germination rate (%)				Average days until germination			
Storage	Wet (x/50)		Dry (x/50)		Wet		Dry	
Hudsonia ericoides	2.0±2.0		11.8±2.2		4±NA		5.9±0.9	
Limonium carolinianum	3.3±1.3		11.3±1.8*		2.3±0.7*		18.6±1.9	
Storage	**Dry**		**Wet**		**Dry**		**Wet**	
Irrigation	**Fresh**	**Salt**	**Fresh**	**Salt**	**Fresh**	**Salt**	**Fresh**	**Salt**
Plantago maritima	39.3±6.4*	6.0±6.0	10.0±3.1	9.3±2.4	3.9±0.9	7.5±1.2	8.1±1.2	13.4±4.5

Notes: This table shows average germination rate and the average number of days until germination ± SE (standard error). The trial used 3 replicates with 50 seeds per replicate. An * indicates that the treatment performed significantly better ($P < 0.05$) than all other treatments. For *Hudsonia ericoides*, 2 of the 3 replicates in the wet treatment did not germinate. For *P. maritima*, the dry storage/freshwater treatment was significantly different from the wet storage/freshwater treatment.

temperate climates preferring cold stratification (Baskin and Baskin 1995, 2014; Fenner and Thompson 2005; Liu and others 2010).

Pre-Storage Treatment: Propagule Preparation

For all berries studied (excluding *G. dumosa*, which did not germinate*)*, seeds separated from the fruit had a significantly higher germination rate. This finding has been reported for *Vaccinium* spp. in the past (Vander Kloet and Hill 2000), but there has been comparatively little work done on *Empetrum* in North America. As berries are usually consumed by predators (Crossland and Vander Kloet 1996) or left to decompose, germination for these species may rely on particular light conditions (Pereira and Mourato 2012), but we did not test light vs. dark treatments in any of these trials. The lack of germination in *G. dumosa* could be caused by this species' association with fire and tendency toward vegetative reproduction (Coladonato 1992).

Stratification Treatment: Moisture

Over the course of 3 trials, 2 storage moisture regimes (freshwater/dry) were tested on 16 species. Out of these species, *C. pickeringii* and *J. gerardii* did not germinate, and *A. margaritacea*, *L. japonicus*, and *S. maritimus* had low germination. The lack of germination in *C. pickeringii* may have been attributable to the duration this species was stored (2 y), as this species has been successfully germinated after a 4-mo storage period (authors' personal observation). As for *J. gerardii*, the lack of germination may have been caused by damage to seeds during storage (for example, by fungal pathogens), as previous studies have successfully germinated this species (Charpentier and others 1998). Although our trial showed low germination of *A. margaritacea* and *S. maritimus* in both the wet and dry treatments, a previous study on *A. margaritacea* reported 47 to 64% germination in dry storage (Flessner and Trindle 2003), and for *S. maritimus* the use of cold, moist stratification improved germination (Clevering 1995). Additionally, germination rate in *L. japonicus* can increase after scarification (Chinnasamy and Bal 2003).

For the most part, species found in a wet environment responded positively to a wet storage treatment. For example, *S. alterniflora, S. patens, S. pectinata, A. glabriusculata,* and *S. sempervirens* all naturally occur in salt marshes (Roland and Zinck 1998), and the germination of these species significantly increased when they were stored wet. The trends observed for the *Spartina* spp. in our study are in line with previous germination experiments conducted with seeds from other regions (Callaway and Josselyn 1992; Bruno 2000; Chung and others 2004).

Previous research indicates that *A. glabriusculata* does not require a dormancy period; instead, germination is inhibited by low temperature, salinity, and the persistent bracteoles that encompass the seeds of this species (Ignaciuk and Lee 1980). Therefore, storage preparation for this species should involve removing seeds from bracteoles and placing them in a moistened container at 4 °C. Research is needed, however, to determine the longevity of *A. glabriusculata* seeds in storage.

For *S. sempervirens,* previous research has been conducted on a variety of this species, *Solidago sempervirens* L. var. *mexicana* L. Fernald (seaside goldenrod [Asteraceae]), which is found in *S. sempervirens*' southern range. Researchers were able to store *S. sempervirens* var. *mexicana* dry for 1 mo at 4 °C and had higher germination than recorded in our study (for both our wet and dry treatment) (Orava and Drake 1997). Based on our study, we recommend cold, moist stratification in freshwater at 4 °C for seeds gathered in Atlantic Canada.

Although no increase in germination rate was observed in any treatments for *F. rubra*, *S. novi-belgii*, or *H. ericoides*, these species did germinate faster in the freshwater storage treatment than in the dry treatment, an indication that cold, moist stratification reduces dormancy. Unlike the previous species, these 3 are not found in coastal wetlands but rather in relatively dry locations within coastal barrens or along the roadsides, which are naturally drier environments. For both *F. rubra* and *S. novi-belgii,* previous studies also show that dry storage is acceptable, with cold-dry stratification the recommended method for germination (Kearns and Toole 1939; Moore and Anderson 2002).

And yet, *O. biennis* had significantly greater germination in the freshwater treatment even though it is not a wetland species. As this species is traditionally found along dry roadside environments, this preference could be attributable to *O. biennis* delaying germination until conditions are favorable for young seedlings. Previous research also indicates that cold-wet stratification results in high germination (95%) for this species (Baskin and Baskin 1994).

Both *P. maritima* and *L. carolinianum* differed in their preferred moisture treatment between the 2013 and 2017 trials, with seeds from the 2013 trial preferring freshwater storage and seeds from the 2017 trial preferring dry storage. This difference is likely caused by the amount of time seeds were stored before germination, with seeds from Trial 2 stored for 2.5 mo and seeds for Trial 4 stored for 2 y. As the germination rate for these species decreased with time in storage, this observation may indicate that these species should be stored wet for short-term storage and dry for long-term storage. A 1972 study involving dry storage of *P. maritima* for several years reported a germination rate similar to our cold, moist stratification treatment (Arnold 1972). However, the difference observed between Trial 2 and Trial 4 may be because of the collection of seeds from different individuals. Future studies testing storage duration and preference should use seeds collected from the same individuals.

Storage Treatment: Temperature

Storage temperature played a significant role in the germination of many of our species, with *S. alterniflora, S. patens, S. pectinata,* and *E. nigrum* preferring storage at 4 °C, a trend consistent with the literature (Bell and Tallis 1973; Seneca 1974; Biber and others 2013a, 2013b). This cooler temperature preference is typical for species from this region and reflects natural winter storage conditions (Baskin and Baskin 2014). Only one species, *E. eamesii,* preferred a colder storage treatment with higher germination observed at –20 °C. Unlike the other species exposed to this treatment, *E. eamesii* is commonly found on exposed rocky outcrops with little shelter from the surrounding environment (Dupuis 1988). This species' preference for –20 °C could reflect some adaptation toward a harsher environment or greater susceptibility to pathogens that can proliferate in unfrozen stratification conditions. A 1986 study also found variation in germination response to temperature in the same two *Empetrum* species examined in this study. Both *E. eamesii* and *E. nigrum* preferred germination at 25 °C; however, at reduced temperatures (5 °C and 10 °C) *E. eamesii* had a higher germination rate than did *E. nigrum* (Dupuis 1988).

Irrigation Treatment

For the 4 species with a saltwater irrigation treatment, *S. patens, S. pectinata, and P. maritima* preferred freshwater to saltwater. For *S. alterniflora,* saltwater did not have a significant impact on germination, and previous studies have also successfully germinated *S. alterniflora* when stored in saltwater (Moorring and others 1971). This pattern reflects where these species naturally occur in the salt marsh, with *S. patens, P. maritima,* and *S. pectinata* found at higher elevations relative to the tidal frame, and *S. alterniflora* found at lower elevations. The trends observed here have been reflected in previous studies, with researchers theorizing that field germination likely depends on rainwater diluting the salt concentrations (Ungar 1978; Shumway and Bertness 1992; Fenner and Thompson 2005; Hanslin and Eggen 2005).

CONCLUSION

For the majority of species examined, germination increased when species were stored in conditions that reflected their natural environment. Therefore, for Atlantic Canada we recommend that species found in wet habitats should be stored in freshwater at 4 °C and species found in dry habitats should be stored dry at 4 °C. As we saw with *O. biennis*, however, species will not always fit this trend. There is still a need to test optimal germination conditions for vegetation in this region. Finally, note that only 9 species had a germination rate greater than 50%. This could be attributable to a preference for vegetative reproduction, or because of a preference for a germination treatment not examined in this study. To address this, future work should examine how light, temperature variation, and storage duration affect the germination of these species.

ACKNOWLEDGMENTS

We thank Erinn O'Toole and Tony Bowron for their assistance with this study. Funding for this project was provided by Chiba University SEEDS Fund (Chiba University Open Recruitment for International Exchange Program), CB Wetland and Environmental Specialists, and the Natural Sciences and Engineering Research Council of Canada through a Discovery Grant and a Vanier Canada Graduate Scholarship.

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Table A.1

Habitats where each species used in this study is commonly found.

Species	Family	Habitat
Anaphalis margaritacea (L.) Benth.	Asteraceae	Roadside
Atriplex glabriusculata Edmondston	Chenopodiaceae	Salt marsh
Calamagrostis pickeringii A. Gray	Poaceae	Coastal barren
Empetrum nigrum L.	Ericaceae	Coastal barren
Empetrum eamesii Fernald & Wiegand	Ericaceae	Coastal barren
Festuca rubra L.	Poaceae	Coastal barren
Gaylussacia dumosa Fern.	Ericaceae	Coastal barren
Hudsonia ericoides L.	Cistaceae	Coastal barren
Juncus gerardii Loisel.	Juncaceae	Salt marsh
Lathyrus japonicus Willd.	Fabaceae	Salt marsh, dunes
Limonium carolinianum (Walter) Britton	Plumbaginaceae	Salt marsh
Oenothera biennis L.	Onagraceae	Roadside
Plantago maritima L.	Plantaginaceae	Salt marsh, coastal barren
Schoenoplectus maritimus (L.) Nels.	Cyperaceae	Salt marsh
Solidago sempervirens L.	Asteraceae	Salt marsh, dunes, coastal barren
Spartina alterniflora Loisel.	Poaceae	Salt marsh
Spartina patens (Ait.) Muhl.	Poaceae	Salt marsh
Spartina pectinata Bosc ex Link	Poaceae	Salt marsh
Symphyotrichum novi-belgii L.	Asteraceae	Coastal barren, roadside
Vaccinium angustifolium Ait.	Ericaceae	Coastal barren
Vaccinium macrocarpon Ait.	Ericaceae	Coastal barren

TABLE A.2

For each trial, this table includes the number of replicates used for each treatment, the number of seeds used in each replicate, and the location in Nova Scotia where seeds were collected.

Trial	Replicates for each treatment	Seeds per replicate	Location of seed collection
1	18, *Vaccinium macrocarpon*	16	44.50722 N, 63.52278 W
	18, *Empetrum nigrum*	8	44.50722 N, 63.52278 W
	18, *Empetrum eamesii*	8	44.50722 N, 63.52278 W
	18, *Vaccinium angustifolium*	41	44.50722 N, 63.52278 W
	9, *Gaylussacia dumosa*	10	44.50722 N, 63.52278 W
2	3	20	44.50722 N, 63.52278 W 44.63222 N, 63.58139 W 44.64556 N, 63.37444 W
3	8	20	44.64556 N, 63.37444 W 44.64972 N, 63.42083 W 44.99722 N, 64.14306 W 45.07889 N, 64.13083 W
4	3	50	44.50722 N, 63.52278 W 44.64556 N, 63.37444 W

Notes: For Trial 1, the number of seeds for each replicate was chosen based on the average number of seeds found in a typical berry for that species.

TABLE A.3

Trial 1: Two-way ANOVA table for the germination rate of E. nigrum, E. eamesii, V. angustifolium, *and* V. macrocarpon *for seeds and berries stored at 4 °C and –20 °C.*

Species	Df	Sum sq	Mean sq	*F* value	Pr(>F)
Vaccinium angustifolium					
Fruit	1	981.6	981.6	45.44	4.14e-09
Temp	1	981.6	981.6	45.44	4.14e-09
Fruit : Temp	1	981.6	981.6	45.44	4.14e-09
Residuals	68	1469.0	21.6		
Empetrum nigrum					
Fruit	1	2813	2812	18.03	6.76e-05
Temp	1	3134	3134	20.09	2.91e-05
Fruit : Temp	1	2812	2812	18.03	6.76e-05
Residuals	68	10608	156		
Empetrum eamesii					
Fruit	1	957	957.0	12.81	0.00064
Temp	1	957	957.0	12.81	0.00064
Fruit : Temp	1	957	957.0	12.81	0.00064
Residuals	68	5078	74.7		
Vaccinium macrocarpon					
Fruit	1	35548	35548	128.4	4.32e-13
Residuals	34	9412	277		

Notes: For *V. macrocarpon*, seeds and berries were stored only at 4 °C.

TABLE A.4

One-way ANOVA tables for the average germination rate for species in Trial 2.

Germination rate	Trans.	Df	Sum sq	Mean sq	*F* value	Pr(>F)
Anaphalis margaritacea		1	2.338	2.3378	3.078	0.154
Residuals		4	3.038	0.7595		
Atriplex glabriusculata		1	7.709	7.709	12.2	0.0251
Residuals		4	2.528	0.632		
Festuca rubra	Log	1	0.01147	0.011473	1.861	0.244
Residuals		4	0.02466	0.006165		
Limonium carolinianum		1	0.6616	0.6616	2.995	0.159
Residuals		4	0.8838	0.2209		
Lathyrus japonicus		1	0.2071	0.2071	1	0.374
Residuals		4	0.8283	0.2071		
Symphyotrichum novi-belgii		1	0.557	0.5570	1.1	0.353
Residuals		4	2.026	0.5064		
Oenothera biennis	Log	1	5.325	5.325	85.86	0.000754
Residuals		4	0.248	0.062		
Spartina alterniflora		1	1.1170	1.1170	9.219	0.0385
Residuals		4	0.4847	0.1212		

(continued)

TABLE A.4 *(continued)*

One-way ANOVA tables for the average germination rate for species in Trial 2.

Germination rate	Trans.	Df	Sum sq	Mean sq	*F* value	Pr(>F)
Plantago maritima	Log	1	5.881	5.881	204.3	0.000139
Residuals		4	0.115	0.029		
Schoenoplectus maritimus		1	0.014	0.0144	0.009	0.929
Residuals		4	6.450	1.6124		
Spartina patens		1	4.629	4.629	35.23	0.00404
Residuals		4	0.526	0.131		
Spartina pectinata		1	7.667	7.667	25.19	0.00739
Residuals		4	1.218	0.304		
Solidago sempervirens		1	9.654	9.654	8.153	0.0461
Residuals		4	4.736	1.184		

Notes: Each species was stored at 4 °C and had a freshwater and dry storage treatment. Non-normal data sets were log transformed (Trans.).

TABLE A.5

One-way ANOVA tables for average number of days until germination for species in Trial 2.

Average days until germination	Trans.	Df	Sum sq	Mean sq	*F* value	Pr(>F)
Atriplex glabriusculata		1	827.2	827.2	40.42	0.00314
Residuals		4	81.9	20.5		
Anaphalis margaritacea		1	2.341	2.3408	4.155	0.178
Residuals		2	1.127	0.5633		
Festuca rubra		1	19.512	19.512	71.82	0.00106
Residuals		4	1.087	0.272		
Limonium carolinianum	Log	1	2.235	2.235	146.5	0.000267
Residuals		4	0.061	0.0153		
Lathyrus japonicus		1	6.0	6.0	1.0	0.374
Residuals		4	24	6.0		
Symphyotrichum novi-belgii		1	295.68	295.68	17.66	0.0137
Residuals		4	66.98	16.74		
Oenothera biennis		1	1872.7	1872.7	92.86	0.000649
Residuals		4	80.7	20.2		
Plantago maritima	Log	1	1.443	1.4431	4.675	0.0967
Residuals		4	1.235	0.3087		
Spartina alterniflora		1	227.55	227.55	13.21	0.0221
Residuals		4	68.89	17.22		
Schoenoplectus maritimus		1	50.58	50.58	0.947	0.386
Residuals		4	213.57	53.39		
Spartina patens		1	3361	3361	39.41	0.00329
Residuals		4	341.0	85.0		
Spartina pectinata		1	2150.8	2150.8	54.06	0.00182
Residuals		4	159.1	39.8		
Solidago sempervirens		1	57.47	57.47	31.07	0.00508
Residuals		4	7.40	1.85		

Notes: Each species was stored at 4 °C and had a freshwater and dry storage treatment. Non-normal data sets were log transformed (Trans.).

TABLE A.6

ANOVA table for the germination rate for S. alterniflora, S. patens, *and* S. pectinata *when stored in freshwater (fresh) vs. saltwater (salt) at 4 °C vs. –20 °C.*

	Trans.	Df	Sum sq	Mean sq	*F* value	Pr(>F)
Spartina alterniflora	Log(1+data)					
Temperature		1	63.5	63.5	59.617	1.47e-10
Irrigation		1	.53	.53	.495	.484
Temp : irrigation		1	1.38	1.38	1.295	.260
Residuals		60	63.91	1.07		
Spartina patens	sqrt					
Temperature		1	141.49	141.49	27.964	1.83e-06
Irrigation		1	16.01	16.01	3.164	0.08033
Temp : irrigation		1	59.6	59.6	11.779	0.00109
Residuals		60	303.59	5.06		
Spartina pectinata	^(1/3)					
Temperature		1	23.36	23.36	22.24	1.48e-05
Irrigation		1	11.82	11.82	11.26	.00138
Temp : irrigation		1	19.65	19.66	18.71	5.84e-05
Residuals		60	63.03	1.05		

TABLE A.7

Two-way ANOVA table for the average number of days until germination for S. alterniflora, S. patens, *and* S. pectinata *when stored in freshwater (fresh) vs. saltwater (salt) at 4 °C vs. –20 °C.*

	Trans.	Df	Sum sq	Mean sq	*F* value	Pr(>F)
Spartina alterniflora						
Temperature		1	47.27	47.27	3.539	.07388
Irrigation		1	118.23	118.23	8.851	0.00722
Residuals		21	280.52	13.36		
Spartina patens	Log					
Temperature		1	0.2982	0.2982	5.207	0.027392
Irrigation		1	1.0247	1.0247	17.894	0.000116
Temp: irrigation		1	0.0842	1.470	0.231782	
Residuals		44	44 2.5198	0.0573		
Spartina pectinata	sqrt					
Temperature		1	14.290	14.290	76.380	6.55e-11
Irrigation		1	4.373	4.373	23.373	1.91e-05
Temp: irrigation		1	1.868	1.868	9.982	0.00297
Residuals		41	7.671	0.187		

TABLE A.8

One-way ANOVA tables for the average germination rate and average number of days until germination for species in Trial 4.

Germination rate	Df	Sum sq	Mean sq	*F* value	Pr(>F)
Hudsonia ericoides	1	0.9176	0.9176	3.499	0.135
Residuals	4	1.0491	0.2623		
Limonium carolinianum	1	3.060	3.060	11.38	0.028
Residuals	4	1.076	0.269		
Days until germination	**Df**	**Sum sq**	**Mean sq**	***F* value**	**Pr(>F)**
Hudsonia ericoides	1	6.380	6.380	1.812	0.311
Residuals	2	7.042	3.521		
Limonium carolinianum	1	570.2	570.2	91.17	0.00067
Residuals	4	25.0	6.3		

Notes: Each species was stored at 4 °C and had a freshwater and a dry storage treatment.

TABLE A.9

Two-way ANOVA test for the average germination rate of Plantago maritima *in Trial 4.*

	Df	Sum sq	Mean sq	*F* value	Pr(>F)
Storage	1	507.0	507.0	7.383	0.02636
Irrigation	1	867.0	867.0	12.626	0.00747
Storage : Irrigation	1	800.3	800.3	11.655	0.00917
Residuals	8	549.3	68.7		

Notes: Treatments included freshwater vs. dry storage and the use of saltwater vs. freshwater irrigation during germination.

AUTHOR INFORMATION

Amy Heim
PhD Student
Saint Mary's University
Halifax, Nova Scotia, Canada
Heim.Amy.E@gmail.com

Ayako Nagase
Associate Professor
Chiba University
Chiba, Japan
anagase@chiba-u.jp

Jeremy Lundholm
Professor
Saint Mary's University
Halifax, Nova Scotia, Canada
Jeremy.Lundholm@smu.ca

Carly Wrathall
Pollution Prevention Officer
Environmental Office, Halifax Water
Halifax, Nova Scotia, Canada
carly.wrathall@gmail.com