

Effects of salinity and temperature on *ex situ* germination of the threatened Gulf of St. Lawrence Aster, *Symphotrichum laurentianum* Fernald (Nesom)

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Abstract

The threatened Gulf of St. Lawrence Aster, *Symphotrichum laurentianum* Fernald (Nesom), is an annual coastal halophyte of the southern Gulf of St. Lawrence, Canada. We examined the effects of salinity (0–20 g/L) and temperature (16–30°C) on germination of *S. laurentianum* seeds over 32 days. The time-course of germination was significantly affected by both salinity and temperature. At lower temperatures (16°C and 23°C), germination was inhibited by salt water at days 16 and 32. However, at 30°C germination rates after 16 days were highest at an intermediate salinity, whereas after 32 days germination was uniformly high in all salinity treatments. Overall, the effect of temperature on germination was much stronger than the effect of salinity. Delays in germination resulting from exposure to salinity or from low soil temperatures could set up strong size asymmetries between seedlings of *S. laurentianum* and the surrounding vegetation, leading to suppression of growing seedlings via shading. Because germination has the potential to be a significant population bottleneck for this seed-dependent annual, conservation efforts should consider microsite suitability for germination in the management of natural populations and in the selection of sites for explants.

Keywords: germination, halophyte, salinity, stress, temperature.

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Introduction

The Gulf of St. Lawrence Aster, *Symphotrichum laurentianum* Fernald (Nesom) (Asteraceae), is an annual halophyte of conservation concern in Atlantic Canada, occurring in salt marshes, dune slacks and other saline habitats in restricted areas of the southern Gulf of St. Lawrence. *Symphotrichum laurentianum* is listed as 'threatened' under Canada's Species at Risk Act and has experienced recent population declines across its range (New Brunswick Department of Natural Resources 2007; Steeves *et al.* 2008; Heard *et al.* 2009). Several large populations persist in the Magdalen Islands of Quebec, but otherwise by 2009 the distribution of the species had been reduced to one population in each of Prince Edward Island (PEI) and New Brunswick (NB) (Paul Ayles, Parks

Canada, PEI National Park, Charlottetown, PEI; Lewnanny Richardson, Piper Project, Rivière à la Truite, New Brunswick, pers. comm., 2009).

Halophytic plants like *S. laurentianum* occupy habitats with an intriguing mix of potentially interacting abiotic and biotic stresses. In addition to the considerable metabolic and ecophysiological cost of salt tolerance (e.g. Konisky & Burdick 2004; Rao *et al.* 2004; Nabity *et al.* 2006; Malagoli *et al.* 2008), salt marshes and similar saline habitats experience extreme temperatures and are also often sites of intense interspecific competition (Ungar 1998; Crain *et al.* 2004; van Wesenbeeck *et al.* 2007). Population-level effects of salinity stress may be particularly likely to arise at the seed germination stage, particularly for annual species, because salinity has strong impacts on germination in many plants including halophytes (e.g. Hanslin & Eggen 2005; Megdiche *et al.* 2007; Meot-Duros & Magne 2008; Easton & Kleindorfer 2009; Li *et al.* 2010). The impact of salinity on germination and growth may also depend on other environmental conditions, and one potential

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interaction of particular interest for halophytes is the interplay between salinity, temperature and interspecific competition. In salt marshes (and similar communities), shading by dominant vascular plants can have major impacts on soil thermal regimes (Bertness & Hacker 1994; Whitcraft & Levin 2007). Temperature effects on seed germination are well known, including a common role for degree-day accumulation in permitting germination in many plant species (Trudgill *et al.* 2005). What is less clear is how often effects of salinity and temperature will be additive versus interacting, and which factor is likely to drive more important variation in germination success for halophytes. The effects of salinity and temperature, if they set the timing of germination and seedling emergence, may in turn influence the impact of interspecific competition on seedlings because germination delays mean stronger size asymmetries between seedlings and surrounding, shading vegetation (Schwinning & Weiner 1998).

For rare or threatened halophytes like *S. laurentianum*, local variation in temperature and salinity (and their interaction) could, via their effects on germination, have important effects on recruitment and hence on population growth and extinction risk. Such variation may arise through features of microsites (e.g. shading, topography, groundwater movement), weather patterns or longer-term climate change, and may present both a threat and an opportunity for managers of threatened halophyte populations. Soils supporting *S. laurentianum* populations are likely to experience salinities varying from 0 (rainwater) to 31 g/L NaCl (seawater inundation), or even higher in hot, dry weather favoring evaporation (Ancheta *et al.* 2010). We are aware of no data for soil temperatures in *S. laurentianum* habitats, but strong spatial, diel and seasonal temperature variations are typical for beach, dune and slack sands.

Houle *et al.* (2001) demonstrated suppression of *S. laurentianum* germination at high salinity, but did not consider temperature or its potential interaction with salinity. Temperature variation may have important consequences for *S. laurentianum* because at maturity the plants are only 5–40 cm tall and tend to occur among other, taller, halophytic species including sedges (*Carex* spp.), cordgrass (*Spartina patens*), bulrushes (*Scirpus* spp.) and arrowgrasses (*Triglochin* spp.) (Jacques Whitford Environment Limited 1994; Lacroix *et al.* 2007). Furthermore, *S. laurentianum* seeds tend to disperse poorly because of sheltering by surrounding vegetation and frequent failure of seeds to detach from seed heads (Lacroix *et al.* 2007). Therefore, seeds do not escape their parents' neighbors and often germinate in partially shaded microhabitats with lower soil temperatures. If low temperatures delay germination relative to surrounding vegetation, strong asymmetric competition via shading is quite likely for *S.*

laurentianum (Reynolds *et al.* 2001). Temperature effects on germination of *S. laurentianum* are also important because of the likely desirability of *ex situ* propagation and reintroduction (e.g. Couillard & Jolicoeur 2008; Heard *et al.* 2009) as part of recovery plans for the species. This will require raising large numbers of *S. laurentianum* seedlings in greenhouses, and knowledge of optimal germination conditions will help maximize recruitment when seed sources (particularly from declining populations) are limited.

We examined the effects of temperature and salinity, and their interaction, on *S. laurentianum* seed germination in the laboratory. Because *S. laurentianum* is an annual, reproduces only from seed and has a limited soil seed bank (Stewart & Lacroix 2001; Kemp & Lacroix 2004) seed germination has the potential to act as a major bottleneck in population growth. Suitability for germination may be an important component of microsite quality for natural *S. laurentianum* populations and for future explants when these are deployed as a conservation tool.

Materials and methods

Experimental protocol

We assayed *S. laurentianum* germination in a 3 × 4 (temperature × salinity) factorial experiment. We harvested filled seed from our greenhouse population of *S. laurentianum*, which was founded from Prince Edward Island seed (Heard *et al.* 2009), and stored it for no more than 24 h in paper seed envelopes at room temperature. We did not cold stratify our seeds. We placed the seeds for the germination trials on 9-cm diameter filter paper (medium porosity; VWR, West Chester, PA, USA) moistened with distilled water or with seawater substitute (Instant Ocean, Spectrum Brands, Atlanta, GA, USA) made up to 5, 10 or 20 g/L NaCl. Seeds on the filter paper were then sealed inside plastic Petri dishes with Parafilm-M (Pechiney Plastic Packaging, Chicago, IL, USA) and the Petri dishes were floated in nine water baths, with three each set to 16, 23 and 30°C. We placed all of our water baths in a growth chamber (Conviron, Winnipeg, MB, Canada) with a 16 h light/8 h dark cycle (to resemble the photoperiod at natural germination in June; Houle *et al.* 2001). Each water bath held two plates of each salt treatment (eight plates in total), and each plate held 20 filled seeds.

We scored the plates for seed germination (emergence of the radicle and cotyledons from within the seed coat) after 16 and 32 days. Plates were checked daily for growth of moulds and mildews, and in the few cases where such growth was detected we carefully cut seeds and seedlings from the mouldy filter paper and placed them on a fresh

Table 1 Generalized linear model analyses of *Symphytotrichum laurentianum* germination

	d.f.	F	P
Germination time-course†			
Temperature	2, 60	60.0	< 0.0001
Salinity	3, 60	3.06	0.035
Temperature × salinity	6, 60	0.82	0.56
Day 16 germinants			
Temperature	2, 60	9.20	0.0003
Salinity	3, 60	0.20	0.90
Temperature × salinity	6, 60	5.20	0.0002
Day 32 germinants			
Temperature	2, 60	143	< 0.0001
Salinity	3, 60	2.59	0.061
Temperature × salinity	6, 60	3.04	0.012

† Increase in the number of germinants between day 16 and day 32 counts. A significant effect is equivalent to a significant time × effect factor in the repeated measures analysis.

plate. We included germinated but mouldy seedlings as successful germinants, even if they later died.

Data analysis

Our experimental design is technically a split-plot (with water bath as the whole plot factor), but in a preliminary split-plot analysis, the water baths accounted for a negligible amount of variance. Therefore, we ignored the allocation of Petri dishes to water baths. Our experiment was then a 3 × 4 (temperature × salinity) factorial with repeated measures of germination at days 16 and 32. Because our data were counts (number of germinated seeds out of 20 in each Petri plate), we fitted generalized linear models in SAS ver. 9.0 (PROC GLIMMIX; SAS Institute, Cary, NC, USA) with binomial errors and a logit link. We began by testing for effects of salinity and temperature on the time-course of germination via analysis of the increase in germination between days 16 and 32. In this analysis, significant main effects are equivalent to time × main effects interactions in a repeated-measures analysis of germination. Following detection of such effects, we fitted separate 3 × 4 factorial models for the day 16 and day 32 data.

Results

The time-course of germination, as measured by the increase in germination from day 16 to day 32, varied strongly and significantly among the temperature and salinity treatments (Table 1a). After 16 days, germination was greatest at our highest temperature, and salinity had complex effects (significant temperature × salinity interaction; Table 1b). At low and medium temperatures, seeds

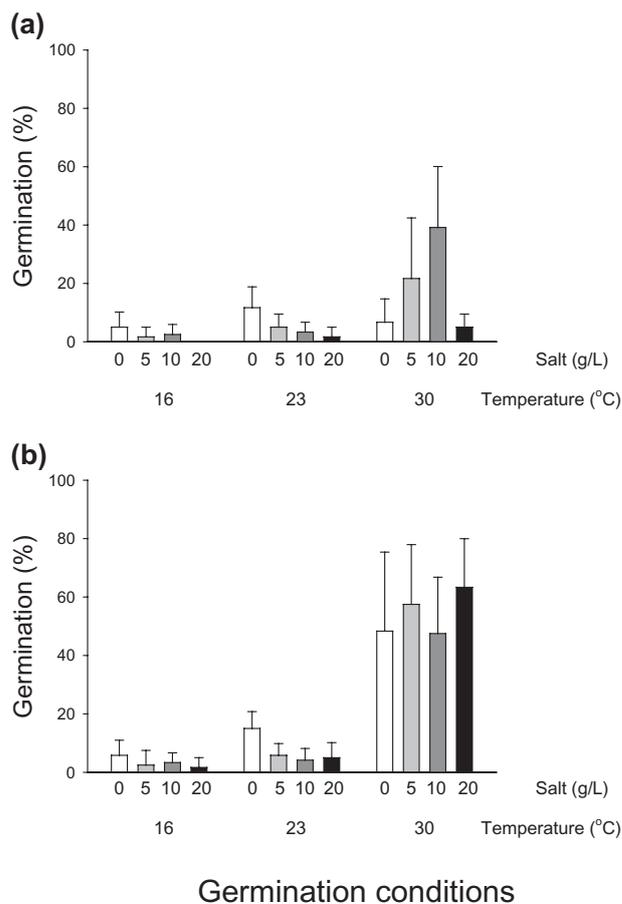


Fig. 1 *Symphytotrichum laurentianum* germination as a function of salinity (g/L NaCl) and temperature, after (a) 16 days and (b) 32 days. Data shown are untransformed. Error bars indicate two standard errors from six replicate plates for each treatment combination. However, the statistical analysis was based on logit-transformed data modeling binomial errors within plates.

germinated best in deionized water, but at high temperature, germination peaked at intermediate salinity (Fig. 1a). After 32 days, however, temperature had by far the dominant effect on germination (Table 1c). A much weaker, although still significant, temperature × salinity interaction arose at day 32 because at low and medium temperatures germination remained highest in deionized water, but at a high temperature germination rates were very high (up to 60%) at all salinities (Fig. 1b).

Discussion

Houle *et al.* (2001) previously assessed the effects of salinity on germination of *S. laurentianum* using a protocol similar to ours, but with only a single temperature treatment (15°C night/25°C day). After 30 days, Houle *et al.* (2001) found the highest germination in zero-salinity

controls and low or zero germination at higher salinity, as did we at comparable temperatures (16°C and 23°C). Our results significantly extend those of Houle *et al.* (2001) by revealing a major (positive) impact of warmer temperatures on germination in *S. laurentianum*. Germination increases with temperature in many species because it is dependent on degree-day accumulation (Trudgill *et al.* 2005), but some plants, including halophytes, show inhibition of germination at temperatures similar to our warmest treatment (e.g. Carter & Ungar 2004). We found no evidence of temperature inhibition for *S. laurentianum*. Dependence on high temperatures for germination may be adaptive for *S. laurentianum* because seeds are produced in autumn, can germinate without cold treatment (Stewart & Lacroix 2001), and must delay germination until spring.

Both Houle *et al.*'s (2001) data and ours indicate inhibition of germination by high salinity at low to moderate temperatures (16–23°C), but we have shown that salinity effects are quite different at higher temperatures. At 30°C, salinity had only a transient effect on germination: at day 16 germination was reduced at both high and low salinity extremes, but by day 32 germination rates were uniformly high across salinity treatments. The inclusion of higher temperatures (our 30°C treatment) revealed that variation in *S. laurentianum* germination as a result of salinity is much weaker than that arising from temperature effects. This suggests that germination in the field will be impacted at least as strongly by microsite temperature as by salinity, and that the impact of salinity under field conditions may not be well predicted by lower-temperature results.

Even transient effects of environmental conditions on germination may have important impacts for *S. laurentianum* if delays in germination slow the emergence of seedlings relative to the growth of surrounding vegetation. Because competition in plants (particularly for light) is frequently asymmetric (Schwinning & Weiner 1998), and because strong shading effects on growth have been documented for *S. laurentianum* (Reynolds *et al.* 2001), a germination delay could lead to *S. laurentianum* seedlings being suppressed by larger neighbors. We have shown that such delays could arise for *S. laurentianum* either as a result of low soil temperatures or high soil salinity.

Houle *et al.* (2001) reported an overall rate of germination considerably higher than we did (> 80% germination in their no-salt control *vs* just 15% and 48% in our 23°C and 30°C controls). Stewart and Lacroix (2001) also measured *S. laurentianum* germination, with no salinity treatments and temperature regimes resembling our 23°C treatment, and reported germination rates consistent with ours (~17%). Differences in seed source may well have contributed to differences in overall germination among

the studies: Houle *et al.* (2001) used seed collected directly from a natural *S. laurentianum* population in the Magdalen Islands, whereas Stewart and Lacroix (2001) used seed from Prince Edward Island (PEI) populations and we used seed from a greenhouse population of PEI origin. Both natural PEI populations and our greenhouse population have much lower genetic diversity than do Magdalen Islands populations and have near-zero levels of heterozygosity (Heard *et al.* 2009). It would not be surprising if inbreeding depression led to reduced germination of PEI and greenhouse seed, something that should be considered as the establishment of greenhouse populations and the deployment of explants from them is explored as a conservation tool for *S. laurentianum* (e.g. Couillard & Jolicœur 2008).

As *S. laurentianum* populations are entirely dependent on seed germination for persistence, and viable seeds do not persist well in seed banks (Kemp & Lacroix 2004), efforts to manage existing populations (and also site selection for new explanted populations) are likely to benefit from the consideration of environmental impacts on germination. Our results suggest that high soil temperatures may be a very important facilitator of *S. laurentianum* germination, and hence population persistence. Vigorous *S. laurentianum* populations sometimes occur on microsites where other vegetation has been removed by disturbance (COSEWIC 2004), and it is possible that this reflects elevated soil temperatures resulting from reduced shading in addition to relaxation of competition. Our results suggest that if managers wish to enhance germination of *S. laurentianum* in the field, providing suitable soil temperatures may be more important than mitigation of soil salinity. Of course, such decisions will need to balance the likely impacts of salinity at other growth stages because high salinity depresses the growth (Houle *et al.* 2001; Reynolds *et al.* 2001), survival and seed set (Ancheta *et al.* 2010) of *S. laurentianum*.

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