

The Effect of Antecedent and Ambient Salinity Levels on Seed Germination in Populations of *Carex lyngbyei* Hornem.

Abstract

The germination responses of four seed populations of *Carex lyngbyei*, an abundant sedge species in the intertidal marshes of the Pacific Northwest, were studied in relation to salinity. Germination only occurred in low-salinity conditions following after-ripening. Pre-germination exposure to high-salinity (20 ppt) conditions produced differential responses in the four populations. Augmentation of germination percentage by pre-germination exposure to high salinity occurred only in seed populations which were exposed to high-salinity conditions in the field prior to spring germination. No change in the speed of germination was observed as a result of exposure to variable salinity levels prior to germination.

Introduction

Carex lyngbyei is a clonal, perennial sedge that occupies a dominant position in estuarine marshes from northern California to Alaska (MacDonald 1977). It is abundant over a broad salinity gradient, from oligohaline to mesohaline, and over a wide elevational range (Jefferson 1975, Disraeli and Fonda 1979, Hutchinson 1982, Ewing 1983). Whereas the wide biogeographic range of a species such as *C. lyngbyei* may simply reflect the availability and accessibility (*via* oceanic dispersal) of suitable habitats, its ecological amplitude may indicate the extent of genetic divergence within the species. Divergence may be visibly expressed by morphological differentiation, or remain veiled, with intraspecific variation restricted to physiological traits. Bimodality of height forms (akin to the well-known case of *Spartina alterniflora* Loisel, dominant in the Atlantic marshes of North America [Anderson and Treshow 1980]) has been described in *C. lyngbyei* populations from several locations (Eilers 1975, Jefferson 1975, Levings and Moody 1976, Ewing 1982), but nothing is known of the physiological tolerances of the species or its subpopulations.

The most widely-examined, and likely most adaptively significant, variable physiological trait between and within maritime plant species is salt-tolerance. Paradoxically, seeds of most halophytic plants germinate best under freshwater conditions and at salinities below 5 ppt. Germination is reduced and delayed at salinities above 10 ppt, and at salinity levels equivalent to those of seawater (36 ppt) germination is negligible, even for

highly salt-tolerant species (Ungar 1982). Therefore, halophytes do not differ markedly from glycophytes in their tolerance to salinity during germination. A more notable differentiating characteristic of halophytes is their ability to remain dormant at high salinities (when immersed in seawater for instance), and then germinate when conditions ameliorate. In addition, exposure to high salinity conditions serves to enhance germination percentages and germination speed in some halophytes (Binet 1964, 1965; Boorman 1967; Lesko and Walker 1969; Ungar and Hogan 1970; Ignaciuk and Lee 1980).

Intraspecific variation in salt tolerance of germinating seeds has been reported for several halophytic plants (McMillan 1959; Wells 1959; Dewey 1960; Cavers and Harper 1967; Workman and West 1967, 1969; Clarke and West 1969; Seneca 1972; Bazzaz 1973; Kingsbury *et al.* 1976; Antlfinger 1981). However, none of these studies deal directly with tolerance of, and response to, salinity conditions during the seed dormancy phase. Differentiation within a population for salt tolerance would likely be most marked in estuarine intertidal species such as *C. lyngbyei*, as estuarine habitats experience much more variable salinity regimes (in space and time) than other coastal environments.

In this and subsequent papers we propose to address the degree of ecotypic variation in *C. lyngbyei*. This study addresses a single question: to what extent is the rate and speed of germination in *C. lyngbyei* populations a function of ambient salinities during seed dormancy and the germination phase?

Materials and Methods

Field Sites

Carex lyngbyei plant material was collected from four sites in the estuarine marshes of southwestern British Columbia (Figure 1). The sites represent contrasting estuarine environments with distinctive annual river discharge and salinity regimes. Two sites were located on the Squamish River delta, situated at the head of a complex estuarine-fjord. The mountainous character of much of the Squamish drainage basin produces a discharge regime with a pronounced summer maximum resulting from snowmelt (Figure 2). In consequence, surface salinities in the estuary during the summer months are much reduced (Figure 3). The magnitude of the seasonal variation across the delta front depends upon distance from the active distributary and the estuarine circulation pattern. Levings *et al.* (1976) present data that demonstrate that surface salinity in the central part of the delta remains elevated (15-20 ppt) throughout the winter and declines rapidly in late spring to <5 ppt.

The Somass River delta is the bay-head delta of the long, narrow fjord known as Alberni Inlet. The Somass River is not primarily snow-fed, and its annual discharge regime more nearly parallels the annual precipitation pattern, with

a mid-winter maximum and later summer minimum (Figure 2). Salinity observations published by Tully *et al.* (1957), Waldichuk *et al.* (1969) and Parker and Sibert (1972) indicate that the surface waters of Alberni Inlet remain brackish for a considerable distance from the delta front, generally remaining below 10 ppt throughout the year within 3 km of the river mouth. Birtwell (1978, and unpublished data) provides comparative information on salinity variation across the delta front (Figure 3b). His results demonstrate that freshwater (<0.5 ppt) conditions persist at the mouth of the Somass River for much of the observation period, whilst oligohaline conditions (<5 ppt) are maintained in the waters of Shoemaker Bay. During this time interval surface salinities in the western estuary were similar to those at Holm Island, for which a more extensive data record exists (Waldichuk *et al.* 1969). These latter data indicate that salinity variations throughout the year are much more uniform than those for the Squamish River estuary, with a maximum in late summer and early fall.

Plant material

Seeds of *C. lyngbyei* were collected in August 1983 from two populations in each delta representing the fresh and saline extremes of the

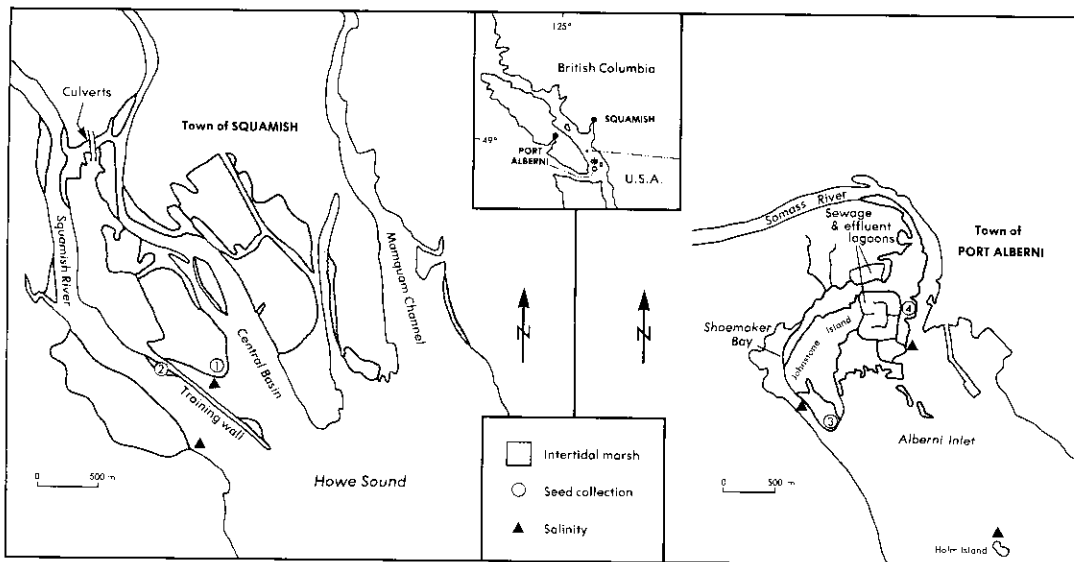


Figure 1. Location of the study sites, seed collection areas, and sites of salinity measurement.

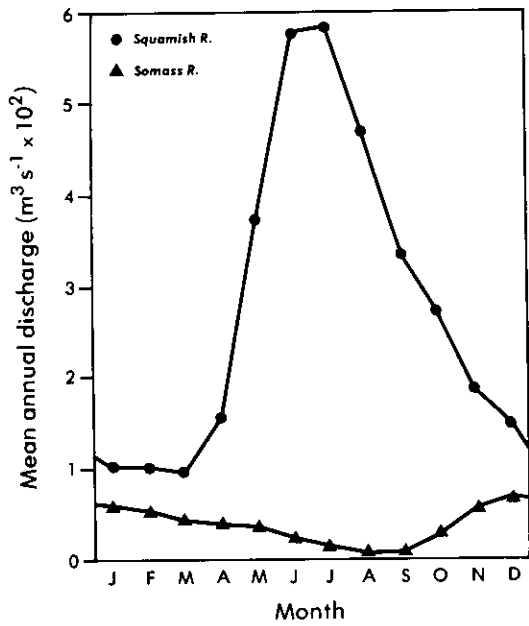


Figure 2. Mean monthly discharges for the Squamish and Somass Rivers. (Source: Environment Canada 1983; data for the Squamish-Cheakamus and Somass-Sproat gauging stations.)

delta-front salinity gradient. At Site 1 (Squamish, "saline") we collected 200 seedheads of *C. lyngbyei* sampled over an area of 1000 m² in a virtually monospecific stand of *C. lyngbyei*. Flowering shoots were relatively infrequent in this salt-stressed habitat, and some 50 percent of the seeds had been eaten by Lepidoptera larvae. Site 2 (Squamish, "fresh") was an 800 m² isolated patch of marsh bordering the main river channel. *C. lyngbyei* was dominant (70 percent cover), with an admixture of *Eleocharis palustris* (L.) R. & S. and *Juncus* spp. One hundred and fifty seedheads were collected over the entire marsh area. Seed production levels were higher, but predation levels were equivalent to those at Site 1.

Site 3 (Somass, "saline") was located in the marsh area adjacent to Shoemaker Bay. The vegetation here was more diverse than at any of the other sites; *C. lyngbyei* accounted for ~60 percent cover. A total of 160 seedheads were harvested over an area of 500 m². Site 4 (Somass, "fresh"), located on the west bank of the Somass River channel, was situated in an extensive

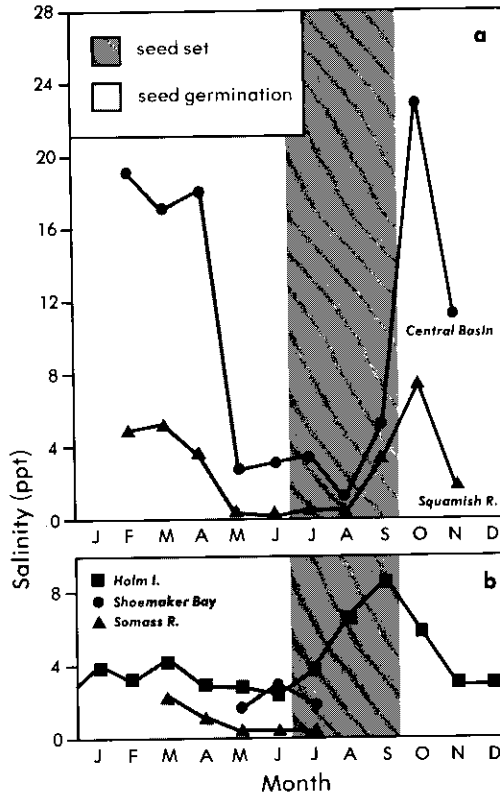


Figure 3. Annual salinity regimes for the a) Squamish and b) Somass estuaries. Data for the Squamish estuary are taken from Levings *et al.* (1976) and those for the Somass from Waldichuk *et al.* (1969) and Birtwell (1978 and unpublished data). Site locations are marked in Figure 1. Periods of seed set and seed germination are indicated.

C. lyngbyei stand with occasional patches of *Scirpus acutus* Muhl. Some 150 seedheads were collected from this locality over an area of 1000 m².

Germination experiments

All *C. lyngbyei* seeds were cleaned, rinsed in undiluted "Clorox" for approximately one minute, and stored dry for 2-3 days. All germination tests were made in 10 cm Petri dishes on two sheets of Whatman No. 1 filter paper moistened with 5 ml of solution. Fifty seeds were placed in an ordered array in each dish. Four replicate dishes were used per population. Dishes were inspected every 3 days and any water loss corrected by adding tapwater to the dishes. Germination (defined

as emergence of the radicle or plumule) was counted and seed removed every 3 days until day 12, then every 7 days. Solutions were produced by adding tapwater to seawater obtained from Burrard Inlet. Salinities were checked using a Y-S-I salinometer.

The salinity pretreatment comprised storage of the seeds in 0 ppt and 20 ppt salinity water in the dark for 100 days at $5^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Seeds were then germinated at three salinity levels (0, 10, 20 ppt) in growth cabinets set on a 12 h photoperiod and $17^{\circ}/8^{\circ}$ thermoperiod. Light in the cabinets was supplied by "cool-white" fluorescent tubes producing $\sim 1200 \text{ W m}^{-2}$ at plant height.

In a further experiment, we attempted to germinate seeds at five salinity levels (0, 5, 10, 15, 20 ppt) without pretreatment.

The germination data were transformed to the arcsin square root of the germination percentage to stabilize the variance. The transformed data were subjected to hierarchical analysis of variance followed by Student-Newman-Keuls "a posteriori" multiple range tests. Mean time to germination (T) was calculated using the formula given by Ignaciuk and Lee (1980):

$$T = \frac{\sum DN}{\sum N}$$

where N is the number of seeds germinating in the interval between the previous count and day D.

Results and Discussion

None of the seeds in the experiment without seed pretreatment germinated. Seeds of *C. lyngbyei*, like those of all its temperate congeners (Andersen 1968), and most other temperate maritime plants, require after-ripening in order to germinate.

In the stratification-salinity pretreatment experiment no germination was observed at salinity levels of 10 ppt or above, and germination percentages in all freshwater treatments were less than 20 percent (Figure 4a). The ANOVA reveals significant differences between deltas, sites and pretreatments in terms of the magnitude of germination in freshwater. Immersion in a 20 ppt solution significantly reduced the germination percentage of seeds from Site 2 and significantly increased the germination percentage of seeds from Site 1 compared to freshwater immersion.

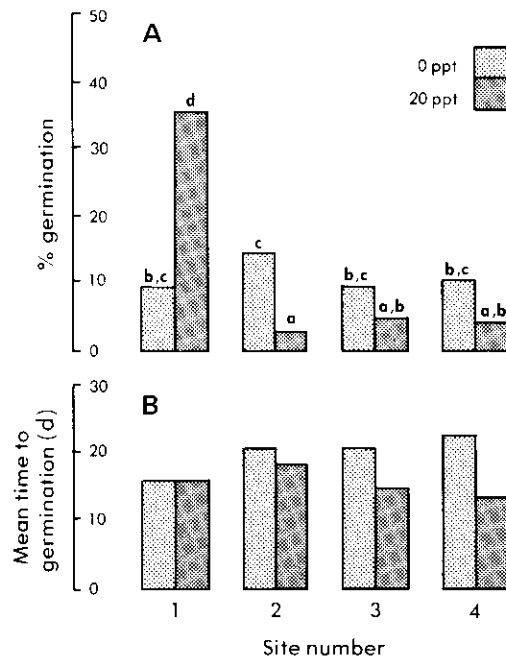


Figure 4. a) Mean germination percentages in fresh water and b) mean germination time in fresh water for seeds from four populations of *C. lyngbyei* following after-ripening in fresh (0 ppt) or saline (20 ppt) water.

In contrast, immersion in freshwater for an extended period of time produced no significant differences between seed populations in terms of germination percentage. Mean time to germination (Figure 4b) was also uniform for all seed populations.

Although *C. lyngbyei* seeds apparently germinate only in conditions of reduced salt stress, there is a strong intraspecific variation in germination response to antecedent conditions. If salinity levels are low during the phase of innate dormancy, all of the seed populations exhibit similar germination responses. However, if salinity levels are high (20 ppt) during dormancy, germination percentages may be augmented, reduced or remain unchanged. The response corresponds to the characteristic salinity regime of the seed provenance. Thus in the environs of Site 1, seawater-dispersed seeds derived from the local population clearly have an advantage over those (such as seeds from Site 2) which are inhibited by exposure to seawater.

TABLE 1. Analysis of variance of percentage germination (arcsin transformed) of *C. lyngbyei* seed from four populations in two deltas as a function of antecedent and ambient salinity.

Source	df	Mean Square	F ratio	Prob.
Deltas	1	0.0276	5.83	0.0175
Sites	2	0.0242	5.09	0.0086
Antecedent salinity	4	0.0331	6.97	0.0001
Ambient salinity	16	0.1609	33.91	0.0000
Error	72	0.0047		

Germination in winter and spring in the field is probably inhibited by low temperatures in all *C. lyngbyei* populations; the size of the germinating seedbank may be primarily a function of ambient and antecedent salinity. Seedling emergence takes place in the field from March to May (Hutchinson, unpublished data), during which time the salinity of the inundating water

declines by an order of magnitude at all test sites. Emergence of *C. lyngbyei* seedlings may coincide with periods of heavy rain during this period; this serves to reduce interstitial salinity still further.

Local differentiation of wind-pollinated plant species such as *C. lyngbyei* does not require geographic discontinuity. Physiological and morphological microdifferentiation has been observed in self-incompatible wind-pollinated species over much shorter distances than those examined here (Jain and Bradshaw 1966, Silander and Antonovics 1979). Such microdifferentiation includes selection at the germination stage for salt tolerant biotypes (e.g. Cavers and Harper 1967); clearly there may be selection for tolerance of salinity conditions antecedent to germination as well.

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