

Final Report:

Germination of water sedge, *Carex aquatilis*, and cotton sedge, *Eriophorum angustifolium*, from Arctic coastal wetlands, Prudhoe Bay, Alaska

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Eriophorum angustifolium, or cottongrass, grows throughout northern Europe, Alaska, Canada, and Siberia.
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Introduction

The Prudhoe Bay oil fields, Alaska were discovered in 1968, and commercial production commenced in 1977 with the completion of the Trans-Alaska Pipeline. Oil production has been declining since 1989, although additional exploratory drilling continues. Support facilities for oil production are built on permafrost soils that surface-thaw in summer to form extensive wetlands composed of moist meadows, sedge marshes, moist sedge-dwarf shrub tundra, grass marshes, small ponds and lakes (Walker and Acevedo 1987). To prevent thawing and subsidence of subsurface, ice-rich soils, gravel pads, 2m (6 ft) or more thickness have been built to support drilling sites as well as roads, airstrips and building pads (Kidd et al. 2006). As well sites are decommissioned, the gravel is wholly or partially removed resulting in the need for site rehabilitation and/or restoration to support wetland plants and, in some instances, enhance wildlife habitat (McKendrick 1991, Jorgenson and Joyce 1994, Kidd et al. 2004, 2006).

Since the 1970s, methods to revegetate arctic wetlands have included a variety of planting techniques, seed treatments, seeding with native and non-native species (mostly grasses), and fertilizer applications (Chapin and Chapin 1980; Bishop and Chapin 1989, Jorgenson 1988, Kidd and Rossow 1998, Kidd et al. 2004, 2006, Maslen and Kershaw 1989, McKendrick 1987, 1991, 2000, McKendrick et al. 1980, McKendrick and Mitchell 1978, Mitchell et al. 1974). Treatments also have included sprigging and plug transplantation (Kidd et al. 2004, 2006), surface manipulation (Streever et al. 2003), as well as natural re-colonization (Ebersole 1987, Schwarzenbach 1996). These methods have been partially successful. The gravelly soils often are dry, nutrient-poor, and have a higher pH and lower organic matter content than surrounding soils, so natural re-colonization does not occur readily (Bishop and Chapin 1989, Jorgenson and Joyce 1994). Methods such as sprigging and plug transplanting are slow, labor intensive and expensive compared to direct seeding. Fertilization, especially with phosphorus, is recommended for long-term survival of plants grown on gravelly sandy soils (BP Exploration and McKendrick 2004).

Two common species in the arctic coastal wetlands are water sedge, *Carex aquatilis* Wahlenb. and cotton sedge, *Eriophorum angustifolium* Honck. *Carex aquatilis* in particular forms large populations that spread vegetatively by rhizomes and often dominate these wetland environments (Shaver and Billings 1975). Despite their abundance, these species have not been considered for revegetation because of poor seed germination and inadequate information on seed development and viability (Dr. William Streever, BP Alaska, pers. comm.). Both *Carex* and *Eriophorum* in arctic environments produce abundant seeds, but seed viability and germination often is low and highly variable among years and locations (Archibold 1984, Billings and Mooney 1968, Ebersole 1989, Gartner et al. 1983).

Germination recommendations for both species vary by location and have included an array of pretreatments such as

light, alternating temperatures, cold stratification, scarification, and high and low temperature dry storage (Amen 1966, Billings and Mooney 1960, Bliss 1958, Hunt and Moore 2003, Johnson et al. 1965, Phillips 1954 and Steinfeld 2001). The purpose of this project was to explore methods of seed germination of *Carex aquatilis* and *Eriophorum angustifolium*, to learn the conditions for germination and dormancy control mechanisms, and identify seed treatments that might enhance germination for eventual use in direct-seeding or plug production for arctic wetland revegetation.

Methods

Seeds were harvested at the Prudhoe Bay oil field on the Eastern Arctic Coastal Plain, Alaska. Seed collection sites for the first germination trials in 2007 were located directly north and east of Prudhoe Bay Operations Center and south of Flow Station 2. Collection sites in 2008 were located parallel to, and on both sides, of a 23 km (14 mi) section of Spine Road between 70.33°N, 148.81°W and 70.32°N, 149.38°W, 10 - 30 m elevation. The harvest areas were within 150m (492 ft) of the road and were representative of moist and wet tundra dominated by sedge species, *Carex aquatilis* Wahlenb and *Eriophorum angustifolium* Honck. (Walker 1985).

In 2007, achenes were collected on seven dates (30 Jul; 10, 20 Aug; 3, 10, 17 and 26 Sep) for *Carex aquatilis* and six dates (excluding final collection date above) for *Eriophorum angustifolium*. Entire heads (composed of one or more spikes or spikelets) were collected into cloth bags, transported to Fairbanks, Alaska, and stored at 15°C for five days. In *Carex*, head length was measured, and achenes were removed by hand rubbing and separation. In *Eriophorum*, achenes were separated by rubbing through a 0.25 mm (60- mesh) soil sieve, then separated using a head thresher (Precision Machine, Lincoln, Nebraska). Data on total number of achenes and filled seeds per head were recorded. For determining filled seeds, the achenes were examined with a lighted dissecting scope and bisected with a razor blade. Three subsamples were taken at random (0.05g each) from all achenes and were dried for moisture content (130-133°C, 24h; International Seed Testing Association [ISTA] 1999, 2003)

Germination tests were conducted with four replicates of 100 randomly sampled filled achenes placed on filter paper, moistened with distilled water in glass petri dishes, and enclosed in plastic freezer bags to reduce water loss. Tests were conducted in growth chambers equipped with cool white fluorescent bulbs (20W and 40W, PAR range 99 - 108 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at seed level). Dishes were rotated within the chambers to avoid location effect. Successful germination was defined as radicle emergence within a 60d period. All trials followed ISTA (1999) protocols for seed testing.

Eriophorum angustifolium seeds were prone to fungal attack and were treated with a single application of Captan fungicide (50% WP; 2.5mg/ml). Achenes that did not germinate were

bisected with a razor blade to determine whether they had been filled. Only filled seeds were included in the statistical analysis (Gartner et al. 1983, 1986). Data were analyzed using arc-sin transformation with analysis of variance, $P \leq 0.05$ or 0.01 , and mean separation by Tukey's HSD (Graphpad Software, Inc. 2007).

First year controlled environment germination experiments included:

- 1) light vs. dark: seeds germinated (60d duration) in exposed (24h light) or foil-covered petri dishes at 10°C constant temperature at all collection dates;
- 2) seed pretreatments: 24h soak in distilled water, 24h soak in 1000 mg/l GA3, or filter paper moistened with potassium nitrate (KNO₃, 0.2%), germination at 15°C constant temperature in 24h light on 10 and 17 Sep collection dates;
- 3) seeds collected on the 10 and 17 Sep collection dates exposed to light at 15° to 30°C, 5°C intervals;
- 4) seeds cold stratified, sandwiched between sheets of moistened filter paper at 4°C for 30, 60, 90, 120 or 150 days on 17 and 26 Sep collection dates, germinated at 10°C in light; and
- 5) seeds collected 3, 17 and 26 Sep and stored 10 mo at -5°C, then germination at 25°C, 25/15°C or 15/5°C alternating temperatures for 15/9h.

In 2008, controlled environment germination experiments included:

- 1) a combination of temperature, light and storage:
 - a. constant vs. alternating temperatures: 25°C or 25/13°C for 15/9h.

b. light vs. dark: seeds were germinated in exposed (24h light) or foil-covered petri dishes under alternating temperatures, 25/13°C for 15/9h.

c. fresh seeds vs. cold dry storage. Seeds in cold storage were enclosed in glassine envelopes, stored dry in glass jars at 4°C (39°F) for 6 months;

- 2) cold stratification or storage (section 1c) followed by cold stratification. Cold stratification treatments as described in 2007 experiments for 60d, in light under alternating temperatures, 25/13°C for 15/9h; and
- 3) harvest date: seeds were collected on 30 Jul, 13, 27 Aug and 10, 23 Sep and germinated in light and alternating temperatures 25/13°C for 15/9h. Seeds harvested on 27 Aug and 10 and 23 Sep were treated with cold stratification, cold, dry storage and a combination of the two (sections 1c and 3).

In 2008, 29 sample sites were selected by species abundance and distance between sites along a transect paralleling the Spine Road. For *Eriophorum angustifolium*, sites to the north and south of the Spine road were separated by at least 500 m (0.3 mi) to prevent multiple sampling of clones. Due to fewer stands with an adequate number of flowering plants, *Carex aquatilis* sites were separated by at least 160 m (0.1 mi) with 11 sites north and 19 sites south of the road.

At each sample site, seed heads were harvested within randomly placed 1 m² (10.8 ft²) plots that were repeated until a minimum of 20 heads were collected from each site. Plant materials were transported to the University of Alaska Fairbanks campus, air-dried at room temperature for 3 - 5d, followed by



Eriophorum angustifolium, growing in northern Europe, image taken 2005.

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hand removal of seeds from the heads and separation of the filled seeds from unfilled seeds and litter with a head thresher. At each site, whole plants were harvested that visually represented the overall maturity stage of the flower/seed heads. Transect data were analyzed using T-test, scatter plots, correlation and regression analysis where appropriate.

Results

Seed Characteristics

The wild-harvested seed heads of *Carex aquatilis* collected in 2007 averaged fewer than 50 seeds per head (composed of one or more spikes), and less than 20 percent of the seeds were filled. More than half of the seed heads contained less than 10 percent filled seeds (Figure 1). The correlation between head length and seed number per head was highly significant, but the linear fit explained only 29 percent of the variability ($P < 0.001$). Head length was not correlated with number of filled seeds per head (Table 1).

Eriophorum angustifolium showed a similar yield of seeds per head as *Carex*, but on average, more than 50 percent of seeds were filled (Table 1). More than half of the seed heads showed 70 percent and greater filled seeds per head (Figure 1).

Moisture content of seeds for both *Carex aquatilis* and *Eriophorum angustifolium* decreased over the collection season with a total of 1.27 percent decrease in *Eriophorum* to 3.07 percent in *Carex* (Figure 2). Active seed dehiscence in *Eriophorum* was observed on the 20 Aug collection date when moisture content was approximately 9 percent. *Carex* spikes did not release seeds through the end of September even with seed moisture content of approx. 7.4 percent (Appendix 1, p. 10).

Controlled Environment Germination Tests

Fresh seeds of both *Carex aquatilis* and *Eriophorum angustifolium* showed less than 3 percent germination under all treatment combinations of light, harvest dates, cold stratification, and temperature in 2007. The common factor for all of these experiments was constant temperature. The chemical pre-treatments of GA3 and KNO3 failed to stimulate germination in either species under constant temperatures as well as light and dark exposures.

In 2008, fresh and stored seeds of *Carex aquatilis* showed significantly greater germination ($P < .01$) in light and alternating temperatures compared to a combination of darkness and constant temperatures (Figure 3). Stored seeds averaged about 10 percent greater germination than fresh seeds. The interaction of all components: storage, light and temperature, were all significant ($P < 0.001$) indicating that the treatments are not independent. For *Eriophorum angustifolium*, only 7 of 2,437 seeds germinated for all combination of treatments. The few seeds that germinated were those treated with high alternating temperatures.

Site comparisons

Our germination analysis of *Carex aquatilis* at the wild sites included 25 of 29 sites because of insufficient seeds at four locations for a minimum of two complete replicates. No significant difference in germination was detected among the sites ($P < 0.05$). Germination percentages of filled seeds ranged from 0 to 88 percent for individual replicates among sample locations, but the overall mean was low (Table 2, page 5). Our examination of scatter plots (Figure 4, p. 6) for data patchiness and trends, evaluation of linear and curvilinear models along

Figure 1. Frequency of seed heads with different percentages of filled seeds for *Carex aquatilis* and *Eriophorum angustifolium*.

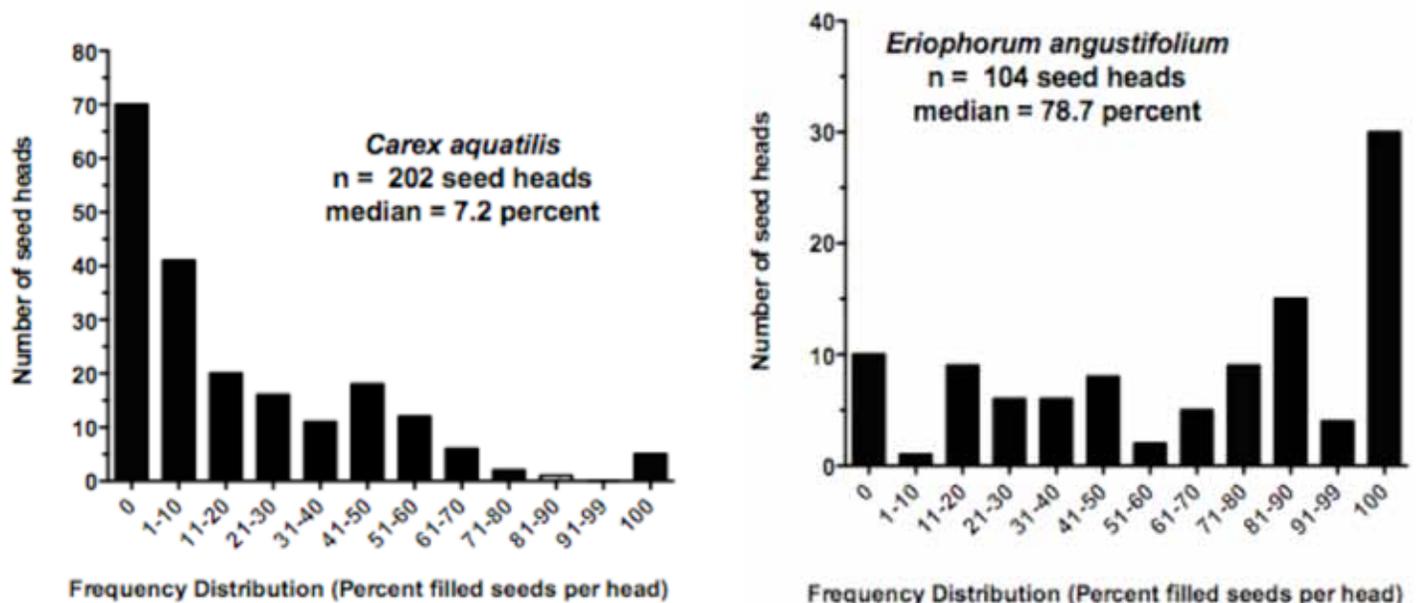


Table 1. Seed characteristics of wild-harvested *Carex aquatilis* and *Eriophorum angustifolium*.

	Minimum	Maximum	Median	Mean + SD	Correlation head length:
<i>Carex aquatilis</i> n=202 heads					
Head length (mm)	5.0	44.0	17	18.4+ 18.37	
Achenes per head	0	181	50	47.4 + 41.2	*** (<.001)
Filled seeds per head	0	60	3	8.7 + 12.0	n.s.
Filled seeds per head (%)	0	100	7.2	18.9 + 24.4	
<i>Eriophorum angustifolium</i> n=104 heads					
Achenes per head	0	44	11	12.9 + 10.5	
Filled Seeds per head	0	27	5	7.3 + 6.4	
Filled seeds per head (%)	0	100	78.7	64.3 + 35.5	

the sampled transect both north and south of the Spine Road showed no pattern of germination that might reveal an effect of site location on germination. Nor were there any sites with consistently higher germination than others that might reveal genetically superior strains. An T-test comparison of all plots north of the road with those to the south did not show significant differences in germination percentages.

Germination began six days after sowing. The speed of germination (days to 50 percent germination, T-50) differed significantly (P<0.05) among sites. Similar to germination

percentages, we found no pattern of germination speed within the sample sites that might show an effect of location on germination or the presence of superior strains. There was no difference in germination speed in sites to the north or south of Spine Road.

Figure 2. Moisture content of wild-harvested *Carex aquatilis* and *Eriophorum angustifolium*, seeds.

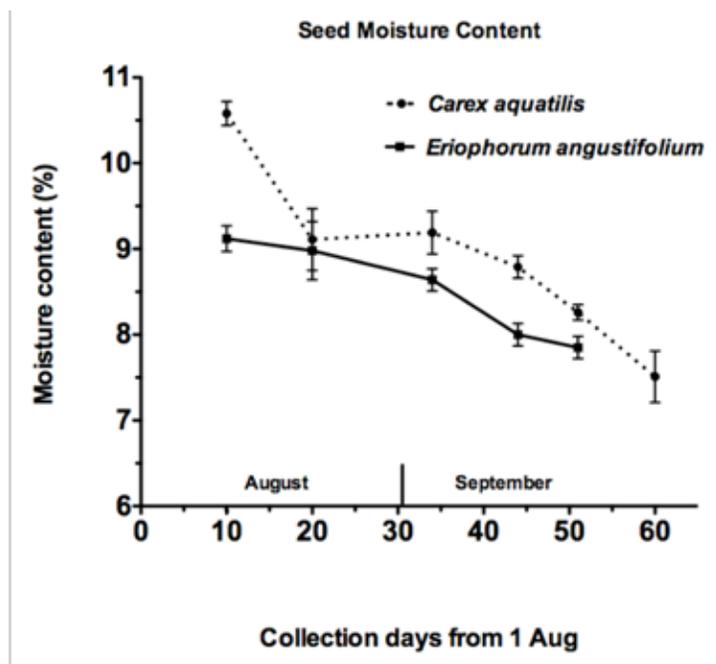
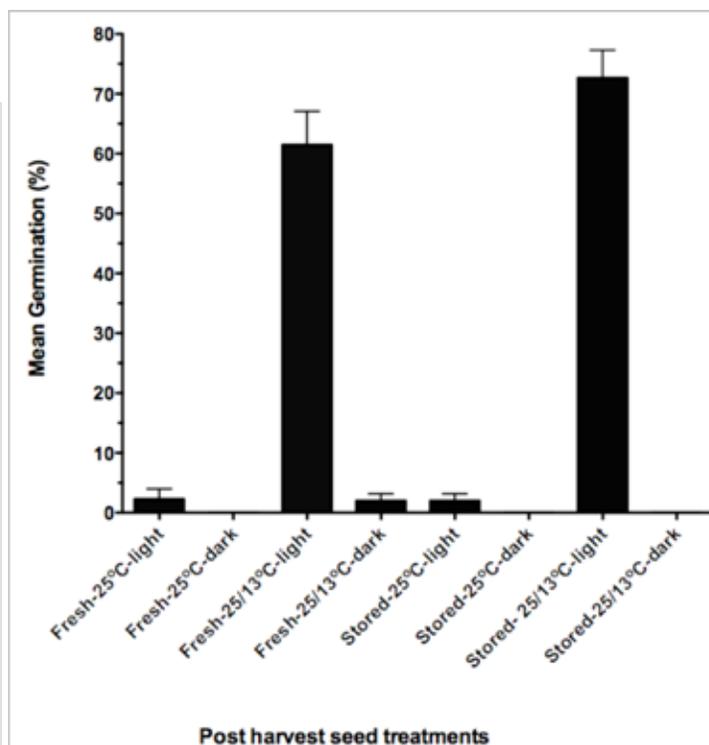


Figure 3. Germination percentages of fresh or stored *Carex aquatilis* seeds treated with light and constant or alternating temperatures.



Discussion

Carex aquatilis produced very few filled seeds in wild populations on the Arctic Coastal Plain. Significant cleaning will be necessary on harvested seeds to separate filled from unfilled seeds prior to direct seeding or controlled environment sowing. *Eriophorum angustifolium* had a high percentage of filled seeds, but cleaning will be necessary to remove the persistent bristles prior to planting for easier handling. Data were collected for one summer season. These species are similar to others where we can expect wide fluctuations in seed yield from year to year (Wiesner et al. 1967). In *Carex*, although head length is correlated with the number of harvested seeds, it is not a reliable predictor of filled seeds. Selectively harvesting the visibly longer heads is not warranted.

Table 2. Germination of wild-harvested *Carex aquatilis* from 25 locations at Prudhoe Bay, Alaska under controlled conditions.

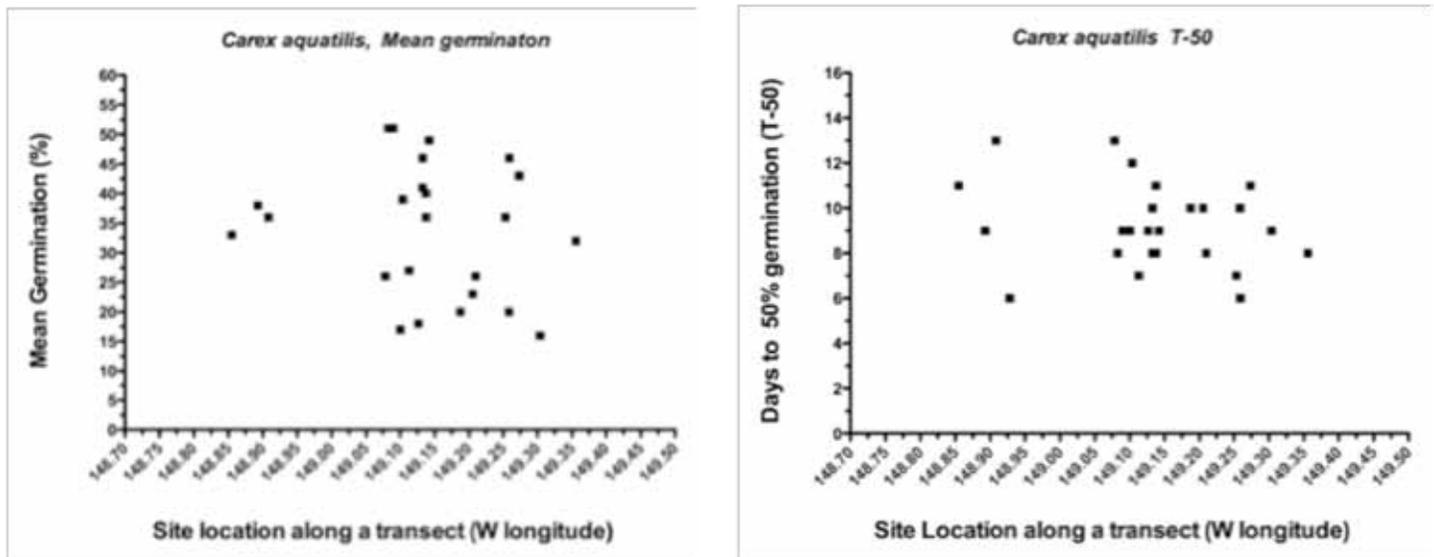
<i>Carex</i>	Germination percentage		
	Minimum	Maximum	Mean (SD)
Site location in relation to Spine Road			
North (n=10)	17	63	34.3 + 13.7
South (n=15)	16	51	35.0 + 12.1
All sites (n=25)	16	63	34.9+ 12.4
	T-50 (days to 50% germination)		
North (n=10)	6	13	9.1 + 2.3
South (n=15)	7	13	9.3 + 1.7
All sites (n=25)	6	13	9.2 + 1.9

For both species, great variation in filled/unfilled seeds occurred across all sites. No patterns occurred to show that selective harvesting of individual clones is warranted. Although we did not detect superior clones, repeated germination tests over several years would be required to identify those with consistently high germination results. It is interesting to note that the median percent of filled seeds for *Carex* was 7.2 percent, while for *Eriophorum*, it was 78.3 percent. Both are wind pollinated species, but perhaps because *Eriophorum* flowers are bisexual, whereas *Carex* has male and female flowers mostly segregated into distinct spikes, *Eriophorum* may have greater pollination success or a greater ability to self pollinate than *Carex*.

Moisture content is routinely used in cultivated agronomic crops as an indicator of seed maturity and a predictor of harvest times. Moisture content in *Carex* and *Eriophorum* declined during the seed-maturation season. *Carex* maturity may also be estimated using color change of the scales and perigynium that progressively change from green to tan to dark brown although not all clones changed color completely by the time the seeds developed a nut-like texture. Crushing the seeds to identify seed firmness stage (milk, dough, etc.) was easy to accomplish. Seeds of *Carex* in early September had a nut-like texture, but germination tests showed they may not be completely mature at that time. Populations of *Eriophorum* dehisce early in the season, and in addition to firmness tests, maturity may be judged by tugging on the bristles.

Ecological differences in vascular plants, mosses, and lichens have been reported in areas covered by dust that blankets the roadsides during the snow-free season because of road traffic and strong prevailing winds from the North (Walker and Everett 1987). Biologists (ABR Alaska pers. comm.) have speculated that flowering and seed production might be influenced by the dust either positively (greater nutrient load from wind-blown soils) or negatively (reduced light levels and photosynthesis).

Figure 4. Scatter plots of germination percentages and speed for seeds of *Carex aquatilis* harvested at 25 wetland locations, Prudhoe Bay, Alaska.



However, we found no difference in seed yield and filled/unfilled seeds on the north (clean) or south (dusty) sides of Spine Road.

We failed to identify optimum germination conditions for *Eriophorum angustifolium*. Despite a wide variety of pretreatments and germination conditions, no combination provided more than 10 percent germination. These results differ from previous research conducted in the British Isles (Pearsall and Wray 1927) where more than 50 percent germination was recorded under a variety of calcium sulfate and pH treatments. Additionally, seeds from the British Isles showed significant variability in germination among collection sites as well as a positive effect of one week cold stratification (4°C). The researchers also noted that viability declined significantly with longer stratification periods as well as dry-stored seeds (Phillips 1954). Research in Germany showed that *Eriophorum angustifolium* seed germination was promoted (max 30%) by alternating temperatures (22/12°C, 14/10h) and treatment with GA3 (0.02-0.1 percent), but was inhibited by cold stratification (Maas 1989). In contrast, polar research (Bliss 1958), showed poor germination following 6 -7 mo storage (-15°C) at constant (22°C) temperatures. More positive results have been recorded with related species, *E. vaginatum* (Gartner 1983, Gartner et al. 1983, Gartner et al. 1986, McGraw 1980), and further efforts with revegetation might emphasize this species rather than *E. angustifolium*.

Carex aquatilis showed promising germination percentages under controlled conditions that warrant further studies for Arctic Slope revegetation. The most important environmental characteristics controlling germination are alternating temperatures and light. These factors are important for germination in a variety of *Carex* species (Amen and Bonde 1964, Bond 1999, Kettenring and Galatowitsch 2007a,b, Kettenring et al. 2006, Leck and Schütz 2005, Schütz 1997a,b, 1998, 2000). One germination protocol recommended removal of the perigynium to stimulate germination (Hunt and Moore 2003), but our study found no benefit to its removal in the dark or light at constant temperatures. Gibberellic acid and potassium nitrate (KNO₃) are frequently used to stimulate germination in dormant seeds (Hartmann et al. 2010), but neither seed treatment improved germination of seeds under constant temperatures.

Alternating temperatures, especially high daytime temperature (>20°C) often is recommended for optimum germination of *Carex* species (Kettenring and Galatowitsch 2007a,b, Kibe and Masuzawa 1994, Leck and Schütz 2005, Schütz 1997a,b, 1998, 1999, Schütz & Rave 1999). We found that *Carex aquatilis* alternating temperatures combined with light are necessary for germination of either fresh or stored seeds.

Contrary to recommendations for other *Carex* species (Budelsky and Galatowitsch 1999), cold, dry storage for at least one winter (6 mo) did not diminish seed germination, nor did it reduce the importance of light and alternating temperatures on germination. Other researchers report the benefits of cold stratification to stimulate germination (Bond 1999, Hunt and

Moore 2003, Hurd and Shaw 1991, Kettenring and Galatowitsch 2007b), but like Steinfeld (2001), we found no such benefit for *Carex aquatilis* germinated under controlled conditions.

In conclusion, we recommend *Carex aquatilis* for further evaluation as a revegetation species for Arctic Slope environments. Germination of seeds under controlled conditions using horticultural plug production is possible on cleaned seeds. Further research will determine if seed germination is possible on gravel pads in situ.

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Carex aquatilis, water sedge, photo taken May 4, 2012.

—Photo by US Forest Service botanist Teresa Prendusi

Appendix 1. Achene/seed maturation phenology of *Carex aquatilis* and *Eriophorum angustifolium*, 2008.

Carex aquatilis

Eriophorum angustifolium

30 Jul

30 Jul



Many plants still flowering, achene development just beginning, perigynium color pale green

Most spikelets intact, scales black, flowering mostly ended; achenes tan

11 Aug

11 Aug



Perigynia and achenes pale green/tan, milk stage (milky solution) when squeezed

Dehiscence was noted as early as 2 Aug

Carex aquatilis

Eriophorum angustifolium

26 Aug 26 Aug



Seeds transitioning from milk stage to dough stage (deforms when squeezed but not milky)

Dehiscence continues

8 Sep 8 Sep



Most plants fully yellow/brown or with slight green mottling; scales dark brown, perigynium/achenes are tan

The only spikelets still attached were located in areas sheltered from wind.

*Carex aquatilis**Eriophorum angustifolium*

24 Sep

24 Sep



Plants fully brown. Most seeds at mature stage, hard, nut-like consistency; scales dark purplish brown; achenes tan to brown

Dehiscence nearly complete, most culms prostrate, a few bedraggled heads visible

seeds

Both: The flower, and later the fruit (achene), is enclosed within a papery sheath, the perigynium. The achene is a dry, one-seeded fruit with an inseparable pericarp. For revegetation, the seed plus accessory tissues (pericarp, perigynium) are planted.



Carex aquatilis inflorescence is a cluster of spikes, the terminal 1-2 spikes are staminate, the lower ones, mostly pistillate, sometimes with stamens at the tips. When mature, the scales are reddish-brown or black. The flowers and later, the achene, are enclosed in a light brown perigynium. Mature achenes are glossy, light brown or tan (additional traits Tande & Lipkin 2003, Ball and Reznicek 2003).

Eriophorum angustifolium bears bisexual flowers in loose, floppy spikelets. Their most distinguishing characteristic is long, white or tawny perianth bristles that remain attached to the fruit upon dehiscence. The scales are black when mature. Fruits are three-angled black or brown achenes (additional traits Ball and Wujek 2003).

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About the Agricultural & Forestry Experiment Station

The federal Hatch Act of 1887 authorized establishment of agricultural experiment stations in the U.S. and its territories to provide science-based research information to farmers. There are agricultural experiment stations in each of the 50 states, Puerto Rico, and Guam. All but one are part of the land-grant college system. The Morrill Act established the land-grant colleges in 1862. While the experiment stations perform agricultural research, the land-grant colleges provide education in the science and economics of agriculture.

The Alaska Agricultural Experiment Station was not originally part of the Alaska land-grant college system. In 1898, the station was established in Sitka, also the site of Alaska's first experiment farm. Subsequent branches were opened at Kodiak, Kenai, Rampart, Copper Center, Fairbanks, and Matanuska. The latter two remain as the Fairbanks Experiment Farm and the Matanuska Experiment Farm. The USDA established the Fairbanks experiment station in 1906 on a site that in 1915 provided land for a college. The land transfer and money to establish the Alaska Agricultural College and School of Mines was approved by the U.S. Congress in 1915. Two years later the Alaska Territorial Legislature added funding, and in 1922, when the first building was constructed, the college opened its doors to students. The first student graduated in 1923. In 1931, the experiment station was transferred from federal ownership to the college, and in 1935 the college was renamed the University of Alaska. When campuses were opened at other locations, the Fairbanks campus became the University of Alaska Fairbanks.

Early experiment station researchers developed adapted cultivars of grains, grasses, potatoes, and berries, and introduced many vegetable cultivars appropriate to Alaska. Animal and poultry management was also important. This work continues, as does research in soils and revegetation, forest ecology and management, and rural and economic development. As the state faces new challenges in agriculture and resource management, the Agricultural and Forestry Experiment Station continues to bring state-of-the-art research information to the people of Alaska.

