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DEL-SG-11-78

GERMINATION OF SEEDS OF SELECTED COASTAL PLANTS

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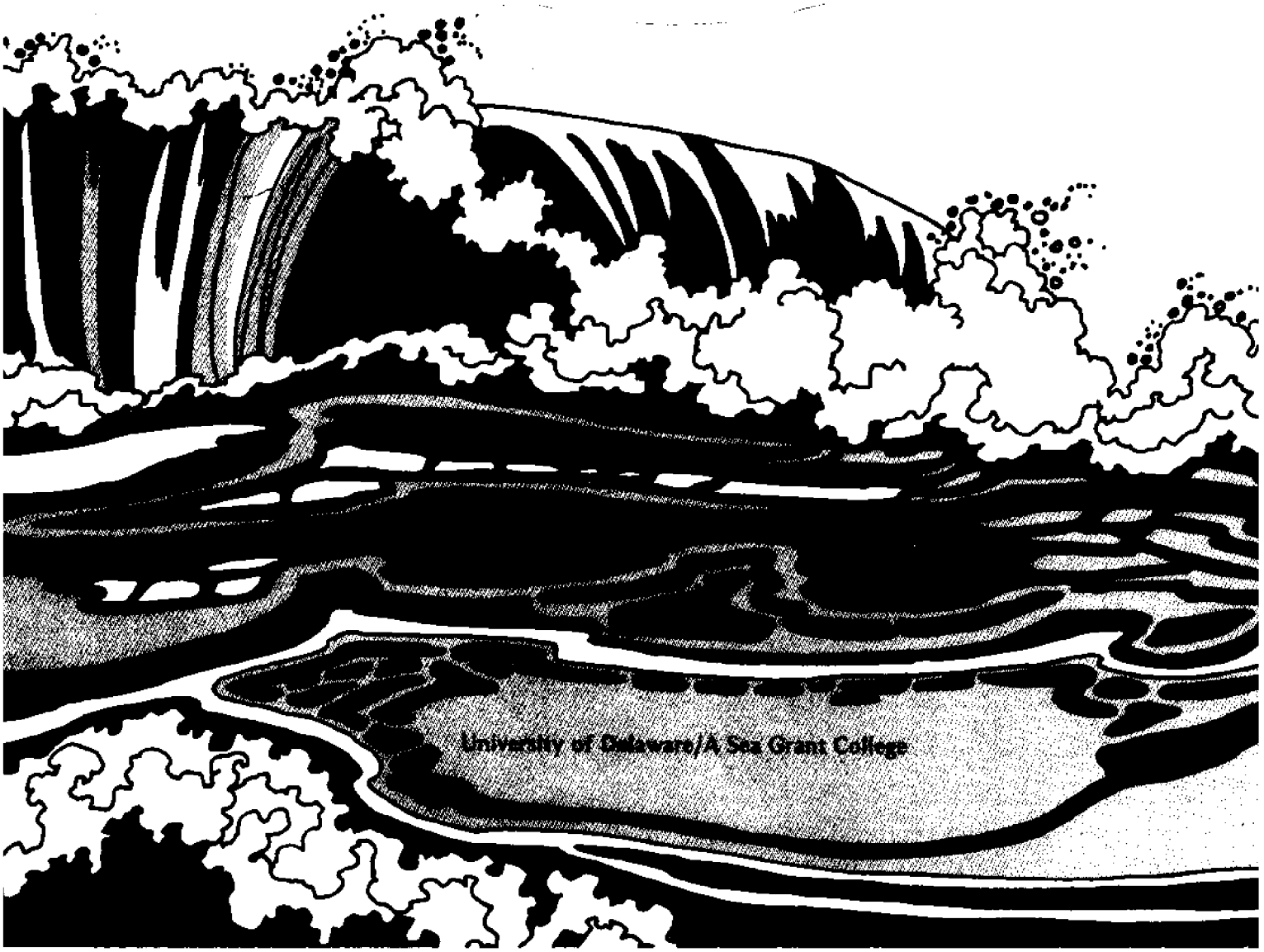

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GERMINATION OF SEEDS OF SELECTED COASTAL PLANTS

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by

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INTRODUCTION

One of the early efforts to find plants which might serve as new food sources for man or feed for domesticated animals and which would tolerate very high salinity (Somers, 1975) was to collect seeds from a variety of sources, especially from coastal areas of the eastern United States. To further evaluate these species, the seeds had to be germinated. The following is an account of various experiments undertaken to produce seedlings.

MATERIALS AND METHODS

Seed sources. All seeds were collected from coastal-plain sites either in tidal marshes or areas adjacent to them, or from coastal sand dunes or adjacent slacks. (See Appendix.) Most seeds were collected in Delaware and Maryland, although some were collected in Virginia and near Nags Head, North Carolina.

Seed storage. Usually, the seeds were stored air dry at 3-4C in an illuminated cold chamber, a standard display cooler such as those used for dairy products. This treatment is to be assumed in the data presented unless other conditions are specified. Commonly, the seeds were held in storage from several weeks to several months before the germination tests. In many cases, storage at room temperature from a few days to a few weeks while the seeds were being threshed, etc., preceded the cold storage.

For the most part, the works of Gleason (1963) and Fernald (1950) were used for species identification after returning specimens to the laboratory. In the field, the works of Moul (1973), Peterson and McKenny (1968), and Petrides (1972) were used.

Germination procedures. For most germination tests, seeds were placed between either germination blotters (Carolina Biological Supply Co., Burlington, North Carolina) or four sheets of filter paper; the blotters and papers were placed in 10 cm x 1.5 cm plastic petri dishes and moistened with either distilled H_2O or artificial seawater prepared from "Instant Ocean" sea-salt mixture (Aquarium Systems, Inc., Eastlake, Ohio). Treatments are indicated under "Conditions" in the tables of results; for example, "1/2 s.w." means 50% seawater. Unless "s.w." is specified under conditions of germination, however, distilled H_2O was used to wet the seeds.

The dishes were placed in reach-in style growth chambers in which light intensity varied from 700 lux to 7500 lux, depending upon such factors as location within the chamber and crowding, and were subjected to various patterns of temperature and light cycling: the two most frequently used

patterns are referred to as "7-27C"--10 hours in darkness at 7C and 14 hours illuminated at 27C, and "13-34C"--10 hours in darkness at 13C and 14 hours illuminated at 34C. The latter cycle was chosen because it had been used to germinate Spartina alterniflora (Garbisch, 1974). Similar thermoperiods were used successfully by Seneca (1974) and Mooring et al. (1971).

Some seeds were germinated in a cabinet with a glass door at room temperature (approximately 25C) and with ambient room light (about 200 lux inside the cabinet). This condition is referred to as "25C." In other cases, germination was at ambient laboratory temperature (about 22C), referred to as "Lab. temp." In some cases the dishes were covered with aluminum foil to exclude light except when they were being examined for germination. This treatment is referred to as "dark."

Some seeds were stratified before germination tests. Usually this was done by placing the seeds between moistened paper in a petri dish and leaving the dishes in the illuminated dairy cooler. Variations of this treatment will be specified.

Scarification of some seeds before germination tests was effected by removing a small portion of the seed and underlying tissue coat with a scalpel or by scratching through the seed coat with a dissecting needle. The location of the incision was random, although it was never near the hilum or micropyle. Another treatment which accomplished the same purpose was to soak the seeds in concentrated H_2SO_4 for varying periods. The acid was washed off thoroughly before placing the seeds for germination.

In a few cases, germination tests were carried out in sand in peat pots which were flooded from the bottom with either H_2O or more or less diluted seawater twice daily. The solution was allowed to drain into containers, corrected for volume loss by adding H_2O, and recycled. These pots were in a room lighted with cool-white fluorescent bulbs supplemented with tungsten lamps. The light intensity was low (6500 lux).

In the petri dishes, protrusion of the radicle beyond the seed coat was used as a criterion of germination; in general, growth continued. However, in some cases in which high salinity was used, such as Strophostyles helvola in 2/3 seawater, little or no growth ensued. In the pots, appearance of the plant above the sand was counted as germination.

In many cases the seeds were treated with 1% NaOCl (20% "Clorox," Clorox

Co., Oakland, California) for 15 to 20 minutes to minimize growth of molds. It later proved that treatment with "Arasan" (thiram) was better.

In some cases, removal of the pericarp or other persistent flower parts facilitated germination. These structures were removed routinely unless otherwise noted.

RESULTS OF GERMINATION TESTS AND COMMENTS

Amaranthus cannabinus. Seeds were collected from Leipsic marsh, Delaware, 10/16/75 and tested 3/11/76 for germination at laboratory temperature. No germination occurred in 27 days.

Ammophila breviligulata. Seeds were collected 10/20/75 and 10/23/75. Germination tests started 11/20/75 with 7-27C cycling.

Dish No.	% Germination			
	Day			
	4	11	18	49
1	0	12	16[a]	28[a]
2	0	12	12[a]	12[a]

[a]Seeds very moldy.

In tests started 12/6/76, stratification between moist filter papers for 31 to 42 days at about 5C in the dark improved germination. Germination was tested in the dark with a temperature cycle of 17 hr at 7C and 7 hr at 29C with H_2O-moistened paper.

Cold treatment	% Germination				
	Day				
	5	7	11	16	18
31 days on moist paper	30	-	33	-	36
	35	-	34	-	37
none	-	14	-	16	-
	-	12	-	16	-

Atriplex patula, var. hastata. Except where specified otherwise, germination was tested with the 7-27C cycling. The seeds were moistened either with H_2O or seawater.

Acquisition No.	Date Collected	Date Tested	Conditions	% Germination Day 14	21	28	35	42	44
102	10/25/74	1/27/75	H_2O	65	-	-	-	-	90
			1/2 s.w.	0	-	-	-	-	90
			1.0 s.w.	0	-	-	-	-	0[a]
		8/13/75	13-34C	35	-	-	-	-	-
			13-34C[b]	50	-	-	-	-	-
102	10/25/74	1/12/76	H_2O	30	60	-	60	60	60
			"	10	70	-	90	90	90
			1/3 s.w.	0	50	-	100	100	100
			"	0	40	-	100	100	100
165	10/26/75	2/3/76	H_2O	100[c]	100	-	-	-	-
			"	90[c]	90	-	-	-	-
165	10/26/75	3/11/76	20C[d]; H_2O	0	20	20	-	-	-
			"	10	30	30	-	-	-
46→212	11/18/75	2/3/76	H_2O	100[c]	100	-	-	-	-
			"	100[c]	100	-	-	-	-
46→212	11/18/75	3/11/76	20C[d]; H_2O	100	100	-	-	-	-
			"	80	80	-	-	-	-

[a] After 72 days, all seeds in full-strength seawater had germinated.

[b] Fruiting bracteoles removed.

[c] Germination had reached this level in 8 days.

[d] Open laboratory, about 20C, no temperature cycling.

In another test, seeds of acq. no. 46→212 were stored for 31 days at 4C in dishes between blotters wet with H_2O. This may have hastened germination; 78% was reached in 4 days. About 12% of the seeds germinated at 4C.

Some conclusions appear evident from these data: the rapidity of germination varies with seed source, and germination is delayed by seawater, but germination can be obtained even in full-strength seawater. Whether or not temperature cycling facilitates germination is not clear. In one case (acq. no. 165), the germination was definitely less and slower in the laboratory at more or less constant temperature. However, with the seed source tested later (acq. no. 46→212), there is little or no difference. Removal of the bracteoles which enclose the fruit apparently facilitates germination.

Borrichia frutescens. Seeds from John Gallagher, in Georgia, were received 11/7/75 and stored dry at 4C. Germination tests were started 3/11/76 using the 7-27C cycling:

Dish No.	% Germination					
	Day					
	5	11	19	27	34	53
1	0	0	0	10	10	10
2	0	0	0	0	0	0

No efforts were made to improve this low germination. Direct seeding into field plots also yielded a low germination. Those plants which germinated the field grew satisfactorily when flooded thrice weekly with estuarine water of about 20 °/oo salinity.

Bromus tectorum.

Acquisition No.	Date Collected	Date Tested	Conditions	% Germination	
				Day	
				14	28
128	7/22/74	1/29/75	7-27C;H_2O	65	90
			7-27C;1/2 s.w.	0	5
			7-27C;1.0 s.w.	0	0
128	7/22/74	8/13/75	13-34C	65	-
			13-34C[a]	90	-

[a]Lemma and palea removed.

The germination of this species appears to be sensitive to salinity. Whether it is only a delayed germination as in A. hastata was not determined. The persistent flower parts apparently reduce germination.

Cakile edentula. In germination tests with this species, distinctions were made between the seeds of the distal and proximal segments of the fruit, and between seeds with pericarp intact and seeds with this structure removed. The fruits were collected 7/31/74 and 8/14/74. Germination tests were conducted in the light except where noted.

Date Tested	Seed	Pericarp	Conditions	% Germination Day 14	28	47
1/24/75	Distal	removed	7-27C;H_2O	50	-	60
			7-27C;1/2 s.w.	5	-	10
			7-27C;1.0 s.w.	0	-	0
		intact	7-27C;H_2O	0	-	0
			7-27C;1/2 s.w.	0	-	0
			7-27C;1.0 s.w.	0	-	0
	Proximal	removed	7-27C;H_2O	30	-	35
			7-27C;1/2 s.w.	0	-	25
			7-27C;1.0 s.w.	5	-	5
		intact	7-27C;H_2O	0	-	0
			7-27C;1/2 s.w.	0	-	0
			7-27C;1.0 s.w.	0	-	0
7/21/75	Distal	removed[c]	7-27C	20	25	-
			7-27C;dark	15	20	-
		removed[b]	13-34C	80	90	-
		removed[c]	13-34C	25	30	-
		intact	13-34C;H_2O	0	0	-
		removed[c]	13-34C;1/3 s.w.	5	5	-
			13-34C;2/3 s.w.	0	0	-
			13-34C;1.0 s.w.	0	0	-
		intact[a]	13-34C	0	0	-
	Proximal	intact[a]	13-34C	0	0	-
		intact	13-34C	0	0	-
		removed[b]	13-34C	55	55	-
		removed[c]	7-27C	25	35	-
		removed[d]	7-27C	35	35	-
		removed[c]	13-34C;H_2O	25	30	-
			13-34C;1/3 s.w.	0	10	-
			13-34C;2/3 s.w.	0	0	-

[a] Pericarp slit lengthwise; end of fruit removed.

[b] Pericarp removed and seed coat ruptured.

[c] Pericarp removed but seed coat intact.

[d] Pericarp removed, seed coat intact, germination in dark.

In this species, the persistent pericarp obviously retarded germination. Merely cutting away a portion of these tissues does not restore germination. An intact seed coat even in the absence of the pericarp appears to retard germination also. The seed in the distal portion of the fruit germinates more readily than that of the proximal portion. There is only a low germination in 1/3- to 1/2-strength seawater and, with one exception of proximal and with pericarp removed, no germination at full strength. Whether the germination was tested in the light or dark seemed to make little or no difference.

Chenopodium album. Seeds were collected 11/3, 11, and 18/75. Germination test was initiated 1/12/76 in petri dishes at laboratory temperature (about 20C). Four dishes each with H_2O and 1/3 seawater.

	% Germination (Mean ± s.e.)			
	Day			
Treatment	7	14	22	30
H_2O	22	30	31	33
	±5.3	±5.8	±5.0	±4.4
1/3 seawater	14	17	17	17
	±4.2	±4.7	±4.7	±4.7

By day 30 the seeds had become very moldy. Observations beyond this date were ignored. Germination in 1/3 seawater was consistently lower than in H_2O.

Chenopodium quinoa. Seeds originally from Bolivia were obtained from J. Clark Ballard, Utah State University, Logan, Utah. Germination tests were conducted with 7-27C cycling except where sand is indicated.

			% Germination		
			Day		
Acquisition No.	Date Tested	Conditions	2	5	36
54	12/3/75	H_2O	80	100	100
	"	1/3 s.w.	70	90	90
58	"	H_2O	80	80	80
"	"	1/3 s.w.	70	90	90
59	"	H_2O	80	100	100
"	"	1/3 s.w.	90	100	100

Chenopodium quinoa (Cont.)

Acquisition No.	Date Tested	Conditions	% Germination Day 2	Day 5	Day 36
61	"	H_2O	60	80	80
"	"	1/3 s.w.	100	100	100
62	"	H_2O	90	100	100
"	"	1/3 s.w	90	100	100
63	"	H_2O	60	60	60
"	"	1/3 s.w.	60	90	90
64	"	H_2O	50	60	80
"	"	1/3 s.w.	40	90	90
66	"	H_2O	90	100	100
"	"	1/3 s.w.	90	100	100
68	"	H_2O	80	90	90
"	"	1/3 s.w.	60	70	70
126	"	H_2O	0	40	80
"	"	1/3 s.w.	10	50	70
			Day 7	Day 11	Day 17
59	1/19/76	Sand; H_2O[a]	0.67±0.67	6.7±1.9	8.0±2.5
"	"	" 1/3 s.w.	0	3.3±1.9	8.0±1.7
"	"	" 2/3 s.w.	0	1.0±0.58	1.67±0.33

[a] 3 pots each; mean ± s.e.

Distichlis spicata. Germination tests were conducted using 7-27C cycling.

Acquisition No.	Date Collected	Date Tested	Conditions	% Germination Day 7	14	21	30	35	42	50	68	78
115	10/11/74	1/29/75	H_2O	-	10	-	-	80[a]	-	-	-	-
			1/2 s.w.	-	0	-	-	40	-	-	-	-
			1.0 s.w.	-	0	-	-	0	-	-	-	-

Distichlis spicata (Cont.)

Acquisition No.	Date Collected	Date Tested	Conditions	% Germination Day 7	14	21	30	35	42	50	68	78
163	10/20/75	1/12/76	H_2O	0	4	16	20	-	44	80	84	100
				0	8	16	32	-	56	68	80	96
			1/3 s.w.	0	4	18	16	-	16	20	32	64[b]
				0	8	12	12	-	20	24	40	72
			2/3 s.w.	0	0	0	0	-	0	0	8	24[b]
				0	0	0	0	-	0	0	0	56

[a]After 126 days, the germination was H_2O, 90%; 1/2 s.w., 100%; and 1.0 s.w., 40%.

[b]After 112 days, the average germination in 1/3 seawater was 96% and in 2/3 seawater 70%.

In another test, seeds of D. spicata were stored for five months in full-strength seawater at 4C. Germination tests were then run using 7-27C cycling for H_2O, and 13-34C cycling for H_2O and 1/3, 2/3, and full-strength seawater. Germination after 14 days was 100% with all treatments. Hence, while seawater delays germination initially, it ultimately is not deleterious after the seeds are subjected to a prolonged treatment in the cold.

Breaking of dormancy of D. spicata by cold treatment (4C in darkness in H_2O) has been reported by Amen, Carter and Kelly (1970). Four weeks of cold treatment was necessary to ensure high germination. Scarification and nitrate were also effective. They consider the dormancy to be the result of an inhibitor of nitrate reductase activity and a restrictive seed coat. The length of the cold treatment in the preliminary experiments here was between 12 to 26 days (exact time, unknown) at 3-5C in artificial seawater and resulted in 80% germination in 35 days at 7-27C in water. This is similar to their result of approximately 85% germination in water after 28 days at 4C followed by 28 days at 24C.

Echinochloa walteri. Seeds were collected near Dewey Beach, Delaware, 10/16/75. Germination tests started 1/23/76 using H_2O and 7-27 cycling.

Dish No.	% Germination: Day 11	19	39	53	67	75	101
1	0	10	10	10	20	30	30
2	0	20	30	40	40	40	40

The seed of this species apparently germinates slowly and, at least this lot, has rather low total germination. Possibly stratification in moist paper in the cold would yield better results.

Elymus virginicus. Lemma and palea were removed before germination tests.

Acquisition No.	Date Collected	Date Tested	Conditions	% Germination: Day 5	11	19	23	40
172	10/28/75	3/11/76	7-27C;H_2O	0	88	88	88	-
				0	44	68	76	-
247		2/18/77	18-29C;H_2O	-	48	-	-	48
				-	68	-	-	68

It appears that this annual plant reaches maximum germination rather quickly.

Euphorbia polygonifolia. Attempts to germinate seeds of this species were mostly unsuccessful, even though the seeds were punctured with a dissecting needle after 7 weeks under germination test conditions. Temperature cycles of 7-27C in the light were used mostly. In only one test was any germination obtained: 10% after 21 days. In this case the temperature cycle was 12-35C, the germination medium was H_2O, and the white layer over the seed coat had been removed.

Hudsonia tomentosa. A maximum of 5% germination of seeds of this species was obtained under any of the conditions used. This success was obtained under the following conditions. First, 2 weeks in continuous dark then light-dark

cycles yielded 5% germination after 44 days. With another seed source, seeds were prechilled in H_2O at 4C for 28 days and germinated at temperature cycles of 12-35C in H_2O; the result was 5% germination after 28 days. But germination of 5% was obtained in 14 days in H_2O with a 12-35C temperature cycle. Obviously, H. tomentosa exhibits a very uncertain response.

Kosteletzkya virginica. Seeds of this species were either scarified by scratching with a needle or soaked in concentrated H_2SO_4 at room temperature to enhance germination. The acid was rinsed off thoroughly before germination tests were initiated using 7-27C cycling and H_2O unless specified otherwise.

Acquisition No.	Date Collected	Date Tested	Pregermination Treatment	Conditions	% Germination				
					Day				
					14	28	48	70	
28	9/12/74	9/23/75	scratched		80	80	80	80	
					-	90	90	90	
			none		50	60	60	60	
					40	40	40	40	
					Day				
					7	10	16		
28	9/12/74	1/14/77	none		52	-	56		
			scratched		-	76	-		
					Day				
					4	18	52	60	98
146	10/1/75	10/3/75	scratched		100	100	100	100	100
					100	100	100	100	100
			none		4	8	16	20	20
					0	0	8	12	16
28	9/12/74	11/20/75	none		28	36	-	-	44
			H_2SO_4;10 min		64	68	-	-	72
					52	56	-	-	76
			H_2SO_4;20 min		80	80	-	-	80
					64	64	-	-	76
			H_2SO_4;40 min		76	76	-	-	76
					88	88	-	-	92
			H_2SO_4;60 min		84	84	-	-	92
					76	76	-	-	76

Kosteletzkya virginica (Cont.)

Acquisition No.	Date Collected	Date Tested	Pregermination Treatment	Conditions	% Germination. Day 7	Day 10	Day 45[b]
146	10/1/75	11/24/75	none		0	0	4[b]
			H_2SO_4;10 min		64	76	92
					88	88	100
			H_2SO_4;20 min		76	88	100
					72	84	100
			H_2SO_4;40 min		80	84	100
					92	92	100
			H_2SO_4;60 min		64	68	90
					76	76	96

Acquisition No.	Date Collected	Date Tested	Pregermination Treatment	Conditions	Day 5	Day 10
146	10/1/75	2/6/76	scratched		78	100
					100	100
					98	100

Acquisition No.	Date Collected	Date Tested	Pregermination Treatment	Conditions	Day 5	Day 36
28	9/12/74	12/8/75	scratched	1/3 s.w.	32	52
					40	68

Acquisition No.	Date Collected	Date Tested	Pregermination Treatment	Conditions	Day 7[a]	Day 14	Day 22	Day 30	Day 42	Day 50
146	10/1/75	1/12/76	scratched	2/3 s.w.	44	44	44	44	44	44
					20	20	20	20	20	20

Acquisition No.	Date Collected	Date Tested	Pregermination Treatment	Conditions	Day 10	Day 20	Day 34
146	10/1/75	3/11/77	scratched	1/4 s.w.[c]	100	100	-
					88	100	-
		3/21/77	scratched	1/2 s.w.[c]	24	-	-
					8	-	-
		1/9/76	scratched	H_2O[c]	12	14	14
				1/3 s.w.[d]	0	0	0
				2/3 s.w.[d]	0	0	0

[a] Seeds and seedlings moldy this day; mold continued.

[b] 20% germination after 100 days; the presence of mold may have influenced the result in the latter part of test.

[c] "Arasan" used to control mold.

[d] In pots of sand.

It is clear that seeds of this species give rather low germination even after 2 years dry storage at about 4C; but very high germination, in some cases 100%, can be elicited even in freshly harvested seeds through damage to the seed coat by scratching or treating with concentrated H_2SO_4. The length of time in the acid is not critical, but 20 minutes may be slightly better than 10 minutes. Even after 1 hour, germination is not greatly reduced. About 1/2 hour appears to be satisfactory.

If scarified, these seeds germinate in petri dishes in the presence of as much as 2/3 seawater, but the germination rate is substantially reduced. Germination in sand in peat pots flooded periodically with H_2O gives poor germination and flooding with diluted seawater gives none.

Lepidium virginicum. Seeds were collected 7/11/74 in Delaware Seashore Park. Germination tests were started 6/25/75 using 7-27C cycling.

	% Germination	
	Day	
Conditions	21	42
H_2O	80	85
1/2 s.w.	75	75
2/3 s.w.	35	90
1.0 s.w.	15	40

Obviously, this species, which is an annual, germinates readily in very saline water.

Limonium sp. The Limonium collected 10/11/74 was originally identified as L. carolinianum (Moul, 1973); however, the calyx around about 40% of the seeds was definitely pubescent, indicating L. nashii (Fernald, 1950). The latter species is considered rare in this area. Some seeds were stored at 4C in H_2O for 28 days or in water or full-strength seawater at 4C for 37 days before germination tests. Some germination tests were conducted in the open laboratory with ambient light and temperature and H_2O or full-strength seawater to moisten the papers.

Date Tested	Pregermination Treatment	Conditions[a]	% Germination Day 14	28	44
1/27/75	none	7-27C;H_2O	5	-	15
		7-27C;1/2 s.w.	0	-	10
		7-27C;1.0 s.w.	0	-	0
3/19/75	none	25C;H_2O	50	50	-
	4C,H_2O,37 days	25C;H_2O	90	90	-
			80	80	-
	4C,s.w.,37 days	25C;H_2O	90	-	-
		25C;1.0 s.w.	0	0[b]	-

[a]Persistent floral parts absent in these tests.

[b]After 14 weeks in full-strength seawater, the seeds were transferred to H_2O; 71% germinated in one additional week.

For L. Humile and L. vulgare, Boorman (1968) reported that removal of the seed coat resulted in rapid germination (data were not given) in fresh H_2O. He suggests that this tissue was responsible for inhibiting germination. Attempts to remove the seed coats of the Limonium used in this study were unsuccessful.

The influence of removing persistent flower parts was examined in additional tests using seeds previously stored 28 days in H_2O at 4C:

Date Tested	Flower Parts	Conditions	% Germination Day 14	28
7/28/75	present	25C;H_2O	20	20
		25C;1/3 s.w.	10	10
		25C;2/3 s.w.	0	0
		25C;1.0 s.w.	0	0
	absent	25C;H_2O	80	80
		25C;1/3 s.w.	20	30
		25C;2/3 s.w.	20	30
		25C;1.0 s.w.	25	38

Obviously, the presence of persistent flower parts decreases germination substantially. Storage in cold H_2O or seawater for a few weeks also enhances germination at room temperature (about 25C) and illumination.

Opuntia humifusa. Seeds of this species were either scarified by scratching with a needle or soaked in concentrated H_2SO_4 to facilitate germination. Cycling of 7-27C was used and germination was in presence of H_2O.

Date Collected	Date Tested	Pregermination Treatment	% Germination Day 70	82	97	118	152
10/28/75	12/2/75	none	0	0	4	8	8
		scratched	0	12	12	16	16
			Day 28	48	56	71	90
	2/3/76	H_2SO_4;15 min	0	30	40	50	50
			0	0	20	40	40
		H_2SO_4;30 min	0	10	10	10	10
			20	40	50	70	70
		H_2SO_4;45 min	0	20	20	20	20
			10	10	20	20	20
		H_2SO_4;60 min	0	0	0	0	0
			10	10	10	20	20

Obviously, the germination of this species is very slow. Moreover, the germination is erratic. The differences between dishes are as large as those between treatments in some cases. It does appear, however, that the intact seed coats retard germination.

Panicum amarulum. Seeds were collected 9/24, 10/20 and 10/23/75 and treated as a single lot. A single germination test was started 11/21/75 in two dishes using 7-27C cycles and H_2O:

% Germination								
Day								
10	17	46	59	66	74	82	94	102
0	0	4	28	44	64	88	96	96
0	8	16	32	60	72	72	74	74

Germination was slow. This seed may not have had sufficient after-ripening treatment.

Panicum miliaceum (P. amarulum?). Seeds were collected 10/1/74. A single germination test was started 1/12/76 in which some seeds were scarified by being scratched with a needle before the test at 7-27C in H_2O.

	% Germination					
	Day					
Conditions	7	14	22[a]	30	42	57
7-27C	0	26	32	36	38	38
	0	18	36	38	44	44
7-27C; scratched	18	24	24	26	28	28
	2	22	22	24	24	24

[a]Seeds were moldy from this day on. Results from this time on should be interpreted with caution.

Panicum virgatum. Very little success was had in germinating seeds of this species. Among several tests with varying conditions, only one resulted in germination: 5% in H_2O.

Salicornia bigelovii. Seeds were collected 10/24/74 and stored at 4C in 1/3 seawater. A germination test was initiated 3/14/75 using 7-27C cycles and H_2O. In 11 days all of the seed had germinated.

Salicornia europaea. Seeds of acq. no. 110 were collected 10/11/74 and some were placed in full-strength seawater in January of 1975; hence, in some experiments they had been in seawater for 5 months at 4C. Seeds of acq. no. 111 were collected 10/25/74 and stored at 4C in 1/3 seawater until 3/20/75, when a germination test was started.

Acquisition No.	Date Tested	Pregermination Treatment	Conditions	% Germination Day 14	% Germination Day 42
110	1/29/75	none	7-27C;H_2O dark	35	40
			7-27C;1/2 s.w.;dark	0	10
			7-27C;1.0 s.w.;dark	0	5
110	7/2/75	4C in s.w.	7-27C;H_2O;dark	80	90
			7-27C;H_2O	100	-
			7-27C;1/3 s.w.	80	100
			7-27C;2/3 s.w.	60	80
			7-27C;1.0 s.w.	75	95
			13-34C;H_2O	85	90
			13-34C;1/3 s.w.	60	60
			13-34C;2/3 s.w.	90	95
			13-34C;1.0 s.w.	95	100
111		4C in 1/3 s.w.	7-27C;H_2O	100	(6 days)

The germination of Salicornia europaea (which had been stored dry in a refrigerator for 32 days) in the dark at 25C in various concentrations of NaCl was studied by Ungar (1962). Germination occurred in up to 5% NaCl. The maximum germination obtained was 40% in H_2O in 30 days, similar to the results of the preliminary experiment reported here. A long period of storage in cold seawater appears to improve germination.

Scirpus rubustus. Seeds were collected 9/18/75. Some were frozen at -20C in an effort to control an insect infestation. One lot was frozen for 3 hours, another lot for 3 hours, thawed, and then frozen for another 24 hours.

Date Tested	Pregermination Freezing Treatment	Conditions	% Germination Day 7	13	25	31	41
9/26/75	none	13-34C;H_2O	-	37	73	73	73
			-	59	72	72	86
	3 hrs	13-34C;H_2O	-	37	73	74	78
			-	39	50	52	52
			-	58	66	67	76
	3 + 24 hrs	13-34C;H_2O	8	31	34[a]	-	-
			3	45	54	-	-
			9	44	55	-	-

Date Tested	Pregermination Freezing Treatment	Conditions	Day 22	30	42	50	57	70	78	98	112
1/12/76	none	7-27C;H_2O	0	0	0	0	0	0	0	0[b]	84[b]
			0	0	0	0	0	0	0	0[b]	60[b]
		7-27C;1/3 s.w.	2 dishes, no germination, 112 days								
		7-27C;2/3 s.w.	"	"	"	"	"	"	"	"	"
		13-34C;1/3 s.w.	0	4	12	16	20	28	28	28	28
			0	0	0	4	4	8	16	20	20
		13-34C;2/3 s.w.	0	0	0	0	0	0	0	4	8
			0	0	0	0	0	0	0	0	0

[a] Dishes dried out; test discontinued.

[b] Temperature cycle changed to 13-34C at 93 days.

Freezing for 24 hours following a 3-hour freeze treatment apparently reduced germination, at least after 25 days. Temperature is important. After 93 days in H_2O with a temperature cycle of 7-27C, no seeds germinated. Changing to a cycle of 13-34C resulted in a high average germination of 72% in another 19 days. At the lower temperature cycle, no seeds germinated in diluted seawater. However, at the higher cycle, an average of 24% germination was obtained in 1/3 seawater after 93 days and eventually a few seeds germinated even in 2/3 seawater.

Setaria geniculata. Seeds were collected 10/16/75. A germination test was started 11/21/75 using 7-27C cycles and H_2O.

% Germination

Day						
48	59	74	94	116	122	164
0	16	32	34	34	34	34
0	12	12	12	12	16	16

These seeds may not have been fully after-ripened.

Setaria magna. Seeds were collected 10/10/74. A germination test was initiated 1/12/76 using 7-27C cycles (3 dishes each treatment) and H_2O. The coats of some seeds were scratched, others were not. After 112 days, 3% of those with unscratched coats had germinated while 14% of those with scratched seed coats had germinated.

Spartina alterniflora. Some seeds of this species were stored in full-strength seawater, others in 2/3 seater in a tightly-closed container at 4C (cf. Stalter, 1972) until germination tests were started in shallow liquid in petri dishes without germination blotters. The seeds were collected 10/28/75 and placed in storage about one month later.

Acquisition No.	Date Tested	Conditions	% Germination Day 7	14	22	30	42	50	64	93	112
203	1/12/76	7-27C;H_2O	0	0	8	20	20	20	20	28	28
			0	4	8	16	20	20	24	24	24
		7-27C;1/3 s.w.	0	0	0	4	8	8	8	12	16
			0	0	4	4	4	8	12	16	16
		7-27C;2/3 s.w.	0	0	4	4	8	8	8	12	16
			0	0	8	8	12	12	20	24	24
		13-34C;H_2O	0	12	16	20	20	20	28	28	28
			0	12	16	16	16	24	24	24	24
		13-34C;1/3 s.w.	4	4	12	12	12	16	16	20	20
			0	4	16	16	16	20	24	28	28
		13-34C;2/3 s.w.	0	0	0	4	4	4	4	8	12
			0	0	8	8	8	8	8	12	16
	3/17/76	13-34C;H_2O	38	92	94	-	-	-	-	-	-
			36	74	92	-	-	-	-	-	-
		13-34C;1/3 s.w.	26	70	90	90	-	90	-	-	-
			24	84	86	90	-	90	-	-	-
		13-34C;2/3 s.w.	14	44	62	64	-	74	-	-	-
			12	56	62	74	-	74	-	-	-
233	1/19/77[a]	13-34C;H_2O	6	52	68	78	-	-	-	-	-
			6	40	66	82	-	-	-	-	-
233-XX	1/19/77[a]	13-34C;H_2O	1	43	56	63	-	-	-	-	-
			2	63	73	-	-	-	-	-	-

			Day 8	13	20
233	1/25/77	13-34C;H_2O	52	82	93
			49	77	89
		7-27C;H_2O	26	67	94
		18-29C;H_2O	6	15	25
			3	9	19
233XX	1/25/77	13-34C;H_2O	61	74	88
			77	89	74
		7-27C;H_2O	25	48	85
		18-29C;H_2O	3	7	14
			4	9	20

[a] Seeds stored in 2/3 seawater.

In another test started 1/25/77, approximately 40,000 seeds of each of acq. no. 233 and 233XX were placed in full-strength seawater in petri dishes with a temperature cycle of 13-34C (10 hours and 14 hours, respectively). The dishes were illuminated during the high-temperature portion of each cycle by fluorescent lamps. The amount of illumination varied considerably because of crowding and mutual shading, and in any case probably never exceeded 3,000 to 4,000 lux. Several hundred seeds germinated. Those which germinated first and grew most rapidly were transplanted into pots of sand and later transplanted into the field for further evaluation.

The tests with acq. no. 203 demonstrate clearly the need for sufficiently long storage to break the dormancy of the seed. In March, relatively rapid, high germination was obtained; but in January, germination was slow, and even after 112 days was only about 25% or less. This species, especially after dormancy has been broken, is not particularly sensitive to salinity. Germination in 1/3 seawater apparently was delayed somewhat but attained as high a total germination as in H_2O. Germination in 2/3 seawater was reduced, but still was relatively high compared with most other species. Germination, however, is facilitated by the appropriate temperature cycling. It was greatly reduced with a 18-29C cycle. A 13-34C cycle appears to be slightly superior to a 7-27C cycle.

Storing seeds of this species poses a problem. Others (Broome, et al., 1974; Mooring, et al., 1971) have found that it does not remain viable if stored dry. However, even in seawater at 4C, much of the seed germinates after several months. In one case, seeds were stored moist in a closed plastic bag at 4C; after 7 months, 45% had germinated.

Strophostyles helvola. Two pregermination treatments were used: storage in H_2O or seawater for 42 days at 4-5C or scratching the seed coat. Germination was tested with temperature cycling or at more or less constant room temperature (about 20C).

Acquisition No.	Date Collected	Date Tested	Pregermination Treatment	Conditions	% Germination Day 14	Day 21	Day 42
97	9/20/74	3/5/75	5C	25C;H_2O	40	-	40
			none	25C;H_2O	0	-	0
				7-27C;H_2O	0	-	0
96	9/23/74	5/14/75	none	25C;H_2O	-	13	13
					-	7	7
			4C;H_2O	7-27C;H_2O	-	0	7
				25C;H_2O	-	20	20
					-	0	0
			4C;1/3 s.w.	25C;1/3 s.w.	-	13	13
			4C;2/3 s.w.	25C;2/3 s.w.	-	0	0
			4C;1.0 s.w.	25C;1.0 s.w.	-	0	0

Acquisition No.	Date Collected	Date Tested	Pregermination Treatment	Conditions	Day 7	Day 38	Day 49	Day 56	Day 112	Day 154
137	9/18-10/8/75	12/1/75	none	7-27C;H_2O	0	10	30	40	50	50
					0	0	20	20	20	20
			scratched	7-27C;H_2O	90	100	-	-	-	-
					100	100	-	-	-	-

Acquisition No.	Date Collected	Date Tested	Pregermination Treatment	Conditions	Day 5	Day 10
137		2/6/76	scratched	7-27C;H_2O	98	100
					84	100
					98	100

Acquisition No.	Date Collected	Date Tested	Pregermination Treatment	Conditions	Day 8	Day 15	Day 21	Day 29
31	9/12/74	3/1/76	scratched	7-27C;H_2O	100	-	-	-
				7-27C;1/3 s.w.	90	100	-	-
32	9/12/74	3/1/76	scratched	7-27C;H_2O	100	-	-	-
					100	-	-	-
				7-27C;1/3 s.w.	90	90	90	90
					90	90	90	90
96	9/23/74	3/1/76	scratched	7-27C;H_2O	96	100	-	-
					100	100	-	-
				7-27C;1/3 s.w.	90	100	100	-
					60	90	100	-

Five additional seed lots were tested for germination in 1/3 seawater using a temperature cycle of 7-27C following scarification of the seed coat by scratching them. All germinated promptly in H_2O: 100% in 7 days. All showed some delay in germination in 1/3 seawater, but gave a high germination eventually: acq. no. 135, 95% ir 20 days and 100% in 28 days; acq. no. 136, 95% in 14 days and 100% in 20 days; acq. no. 141, 95% in 20 days; acq. no. 142, 100% in 7 days (no delayed germination); and acq. no. 173, 90% in 14 days and 100% in 20 days.

It appears that if the seeds are not either chilled in H_2O or dilute seawater or scarified, they germinate poorly, even after prolonged dry storage at 4C. Storage at 4-5C in H_2O or 1/3 seawater for 6 weeks elicits some germination; scarification elicits prompt and complete germination. Even in 1/3 seawater, germination is essentially 100% following this treatment, but is delayed somewhat in most cases.

While this species germinated well in 1/3 seawater, seedlings transplanted into pots of sand and flooded thrice weekly with 1/3 seawater in an illuminated growing room did not grow well for the most part. Observations 6 weeks after transplanting showed acq. no. 147, 68% dead; acq. no. 146, 64% dead; acq. no. 32, 26% dead; acq. no. 142, 56% dead; and acq. no. 141, 76% dead.

Uniola paniculata. Seeds were collected 9/21/74. No success was had in germinating them without previous storage in H_2O at about 5C. (Scarification was not used in this case.) Germination tests were conducted using H_2O.

Date Tested	Pregermination Treatment	Conditions	% Germination, Day 14	% Germination, Day 28	% Germination, Day 42
3/12/75	30 days;5C,H_2O	7-27C	0	10	-
			0	0	-
6/4/75	41 days;5CH_2O	12-35C;dark	17.7	-	19.3
		(6 dishes)	±2.81	-	±3.85
		12-35C;light	23	-	27
			16	-	19

It appears that germination is not very sensitive to illumination.

Zizania aquatica. Seeds were collected 9/25/74. A germination test was initiated 2/5/75. The seeds were suspended in H_2O or seawater at 25C rather than being placed between moist paper.

	% Germination	
	Day	
Conditions	14	28
H_2O	75	75
1/3 s.w.	5	5
2/3 s.w.	0	0
1.0 s.w.	0	0

Seeds of this species eventually germinated at about 4C (in dairy cooler) in H_2O. Germination at these temperatures apparently occurs naturally in Minnesota in April (Oelke, et al.). A similar phenomenon occurs along tidal streams in Delaware while the water is still cold.

Because the Zizania aquatica seed had been stored since collection in cold H_2O in the refrigerator, it had received over 4 months of cold treatment prior to the preliminary experiments. Oelke, et al., reported the necessity of 3 months of cold treatment for germination. Although the temperature used for germination was above the optimum of 63F (it was approximately 77F), germination was greater than 70% in 2 weeks. Such seed is considered to be of high quality (Oelke, et al.).

DISCUSSION

The rather poor germination of some species may have been the result of inadequate after-ripening. Dry storage at about 4C probably is not adequate for some seeds. However, no systematic study of after-ripening treatments was made. In other cases the seed may have been immature when collected. Frequently one is faced with a dilemma when collecting seeds of wild species: waiting until they are ripe yields no seeds because they shatter and fall to the ground almost as soon as they ripen, yet taking them earlier yields immature seeds.

Examples of enhancement of germination by treatment which would be expected to promote after-ripening are stratification of seeds of Ammophila breviligulata between moist sheets of paper at about 5C, or storing in H_2O at this temperature; storage of seeds of Distichlis spicata in seawater at about 4C; soaking of seeds of Limonium carolinianum/nashii in H_2O or seawater at about 4C; and storage of seeds of Salicornia europaea in seawater (1/3 or full strength) at about 4C, although such a treatment was not successful with seeds of Hudsonia tomentosa.

Seneca (1969) and Westra and Loomis (1966) were able to obtain far better germination of Uniola paniculata from North Carolina than we obtained in this study. One possible explanation for the differences is that temperatures during dark periods of both these studies were higher than those in ours; furthermore, light periods of Seneca's study were shorter (7 hr ± 1 hr) and thus dark periods were longer than those in this study. Another explanation for the differences is that the germination of U. paniculata varies greatly with seed lots (Seneca, 1969). Finally, Westra and Loomis (1966) demonstrated the presence of a growth inhibitor in the endosperm in this species. In addition to stratification followed by either an alternating or by a constant thermoperiod with a high dry temperature (30C), dormancy may be overcome by cutting into or exposing the endosperm (Westra and Loomis, 1966).

The rather large difference in germination between duplicate dishes in some cases was disturbing. No explanation was apparent, unless it reflects an inherent variability in the seeds. Small numbers of seeds, in some cases only 10, may have provided an inadequate sample. However, no attempt was made to resolve this issue in most cases. For our purposes , precise quantitative data were not needed in such cases.

It is clear that a persistent pericarp or other floral parts inhibit the germination of some of the seeds tested, as with Cakile edentula, Bromus tectorum (?), and Limonium carolinianeum/nashii. In other cases the seed coat itself inhibits germination, as with Cakile edentula, Kosteletzkya virginica, Opuntia humifusa, and Strophostyles helvola. At least in the case of K. virginica there seems to be little or no seed dormancy not related to the intact seed coat. Freshly harvested seeds germinate promptly after treatment with H_2SO_4.

It appears that seeds of some species which are annuals germinate more readily than those of many perennials. Compare Atriplex patula, Bromus tectorum, Cakile edentula (after removal of pericarp), Chenopodium album, Ch. quinoa, Elymus virginicus, Lepidium virginicum, Salicornia bigelovii, S. europaea, Strophostyles helvola (after scarification), and Zizania aquatica, all annual species, with the following perennials: Ammophila breviligulata, Borrichia frutescens, Hudsonia tomentosa, Opuntia humifusa, Panicum amarulum, P. miliaceum (P. amarulum?), Rosa rugosa, and Uniola paniculata.

Rapid germination may contribute to maintaining a persistent population in the case of an annual. It would help to keep its niche occupied. On the other hand, a perennial is not faced with reoccupying its niche each year. The following species show clearly that a difference in germination rate does not always distinguish annuals from perennials: Kosteletzkya virginica, a perennial which germinated readily following scarification; Scirpus robustus, a perennial which germinated readily after the seeds were frozen, but not without such a pregermination treatment; Distichlis spicata and Spartina alterniflora, perennials which germinated readily after storage in more or less diluted seawater at about 4C; and Limonium sp., a perennial which germinated readily after storage at about 4C in H_2O or seawater. Such observations indicate that the difference between annuals and perennials may be related to after-ripening requirements.

It is obvious that a number of these coastal species (and the inland Chenopodium quinoa) will germinate in diluted seawater, though the germination is frequently delayed or reduced in amount. In view of the success of Epstein and colleagues (1977) in selecting salt-tolerant strains of barley from a population with inherently less initial salt tolerance than the ones in this study exhibit, it is likely that highly salt-tolerant strains could be selected from the following species: Bromus tectorum, Cakile edentula, Chenopodium album, Ch. quinoa, Kosteletzkya virginica, Lepidium virginicum, Limonium sp., Scirpus robustus, Strophostyles helvola, and Zizania aquatica.

Some species already exhibited a high level of salt tolerance during germination. For example, Atriplex patula var. hastata in other studies (unpublished) achieved good germination in the field with irrigation water of 30 to 32 ^{o}/oo salinity). Distichlis spicata achieved 100% germination in 35 ^{o}/oo salinity after the seeds had been stored for 5 months in full-strength seawater (35 ^{o}/oo salinity). Spartina alterniflora seeds have been selected in other studies (unpublished) for germination at 35 ^{o}/oo salinity following storage for several weeks in 2/3 seawater. Mooring et al. (1971) report a maximum tolerance limit for germination between 6% and 8% NaCl.

Appendix

SEEDS USED IN GERMINATION TESTS

Unless otherwise specified, seeds were stored air dry in paper envelopes at about 4C in a commercial-type cooler such as used to display dairy goods for retail sales (storage condition "4C"). This cooler was illuminated with a 40-watt fluorescent lamp which was left on continuously and by ambient room light through the glass doors. Some of the seeds collected in 1974 were stored in glass jars at ordinary room temperature and illumination until January 1975 and were then moved to the cooler. These lots are identified as "1975-4C."

Species	Acquisition No.	Source	Date Collected	Storage Conditions
Amaranthus cannabinus	164	Tidemarsh, Leipsic, DE	10/16/75	4C
Ammophila breviligulata	175	Sand dunes, Cape Henlopen State Park, Lewes, DE	10/23/75	4C
Atriplex patula, var. *hastata*	102	Savage's Ditch, DE Seashore Park	10/25/74	1975-4C
	165	Border of plot sprinkled with diluted seawater, summer 1975, Lewes, DE	10/16,28 & 11/3/75	4C
	46→212	Plot sprinkled with diluted seawater, summer 1975, Lewes, DE	11/18/75	
Borrichia frutescens	170	John Gallagher, Sapelo Island, GA	Fall 1975	4C
Bromus tectorum	128	Cape Henlopen State Park, Lewes, DE	7/22/74	4C[a]
Cakile edentula	98a 98b	Beach, Cape Henlopen State Park, Lewes, DE	7/31, 8/14/74	1975-4C
Chenopodium album	201	Border of plots sprinkled with diluted seawater, summer 1975	11/3,11, 18/75	4C
Chenopodium quinoa	54,58, 59,68, 126	J. Clark Ballard, Utah State Univ., Logan, UT (Seeds originally from Bolivia)	Received 11/25/74	4C

Species	Acquisition No.	Source	Date Collected	Storage Conditions
Distichlis spicata	115	Tidemarsh, Indian River Inlet, DE Seashore State Park	10/11/74	1975-4C[b]
	163	Canary Creek Marsh, Lewes, DE	10/20/,23 11/3/75	4C
Echinochloa walteri	161	Roadside ditch Dewey Beach, DE	10/16/75	4C
Elymus virginicus	172, 247	Hedgerow at high-tide level, Canary Creek Marsh, Lewes, DE	10/28/75	4C
Euphorbia polygonifolia	99	Beach, Tower Road, DE Seashore State Park	10/4/74	1975-4C
	117	Beach, Dewey Beach, DE	9/11/74	1975-4C
Hudsonia tomentosa	123	Dunes, Cape Henlopen State Park, Lewes, DE	7/14/74	4C[a]
Kosteletzkya virginica	28	Tide marsh, "Twin Bridges," Hwy 9, near Silver Run, DE	9/12/74	4C in H_2O
	146	"	10/1, 10/75	
Lepidum virginicum	121	DE Seashore State Park	7/11/74	4C[a]
Limonium carolinium?, nashii?	119	Tidemarsh near Indian River Inlet, DE Seashore State Park	7/11/74	1975-4C
Opuntia humifusa	162	Sand, back of barrier dune, Lewes, DE	10/28/75	4C
Panicum amarulum	157	Dunes, Cape Henlopen State Park, Lewes, DE	9/24, 10/20, 23/75	4C
Panicum miliaceum (P. amarulum?)	44	"	10/1/74	4C
Panicum virgatum	118	Back of dunes, near Dewey Beach, DE	9/11/74	1975-4C
Rosa rugosa	103	Near campground, Cape Henlopen State Park, Lewes, DE	8/6/74	1975-4C
Scirpus robustus	140	Roadside ditch, edge of marsh, Oyster Rocks, DE	9/18/75	4C
Setaria geniculata	159	Along roadside, south of Dewey Beach, DE	10/16/75	4C
Setaria magna	47	Along roadside, Hwy 9, near Flemings Landing, Kent Co., DE	10/10/74	4C

Species	Acquisition No.	Source	Date Collected	Storage Conditions
Spartina alterniflora	203	Banks of Broadkill River, Lewes, DE	10/28/75	4C in 2/3 seawater
	233	Canary Creek Marsh, Lewes, DE	10/26, 28/75	"
	233XX	Same as 233; selected for larger inflorescences	"	"
Strophostyles helvola	31	Bank of dredge spoil, Pilot Town Rd., Lewes, DE	9/12/74	4C
	32	Edge of upper marsh, Lewes, DE	9/12/74	4C
	96	Back of barrier dune, Nag's Head, NC	9/23/74	1975-4C
	97	Savage's Ditch, DE Seashore State Park	9/21/74	1975-4C
	135	Sand dunes, near Coast Guard Dorm, Lewes, DE	9/12/75	4C
	136	Dredge spoil bank, Pilot Town Rd., Lewes, DE	9/16/75	4C
	137	Roadside, Hwy 9, Leipsic, DE	9/9,18, 10/8/75	4C
	141	Fresh water plot, summer 1975, Lewes, DE	9/29/75	4C
	142	Fresh water plot, summer 1975, Lewes, DE	9/29/75	4C
	173	Dune, near Pollution Ecology Lab, Lewes, DE	11/3,11/75	4C
Uniola paniculata	95	Dune, Nag's Head, NC	9/21/74	1975-4C
Zizania aquatica	101	Marsh, Riverside Drive, Salisbury, MD	9/25/74	4C[c]

[a]Stored air dry in glass jar in a home refrigerator until January 1975 and then placed in the dairy cooler.

[b]Stored air dry at room temperature until January 1975 and placed into full-strength seawater at 4C.

[c]Stored in H_2O in an ordinary refrigerator until January 1975 and moved to the dairy cooler where storage in H_2O was continued (cf. Oelke, Elliot, Kernkamp and Noetzel).

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