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EFFECTS OF SALINITY ON GAMETOPHYTE GROWTH OF *ACROSTICHUM AUREUM* AND *A. DANAEIFOLIUM*

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ABSTRACT

Spore germination and gametophyte growth under salinity regimes varying from 0.0 to 3.0% NaCl was studied to determine the stress tolerance of the gametophyte generation. Responses of both New World species of *Acrostichum* are similar to those of other mangrove species which have been studied. *A. aureum* has slightly greater tolerance to increased salinity than *A. danaeifolium.* Growth responses of *A. aureum* suggest it can be classified as a true halophyte, whereas those of *A. danaeifolium* suggest it is a semi-halophyte. The response of the gametophyte generation of these species to salinity parallels the natural habitats of the sporophyte generation.

INTRODUCTION

Halophytes are notably rare in pteridophytes. The most well known example is the mangrove fern genus *Acrostichum.* There are three species circumscribed in this genus, each with different apparent tolerance to salinity. The observed habitat variation of the species forms a continuum from fresh water to inundation by tides and sea water. The most widely distributed species is *A. aureum* L., circumtropical in distribution and frequently forming large colonies in mangrove swamps, salt- and brackish-marshes, and low hammocks near sea water (Holttum 1955; Small 1938; Walsh 1974). The remaining two species are much more restricted in distribution. In tropical Asia and Australia, *A. speciosum* Willd. occurs in mangroves (Walsh 1974) frequently inundated by tides and has a greater tolerance for sea water than *A. aureum* (Holttum 1955). *Acrostichum danaeifolium* Langsd. & Fisch. is distributed in the New World tropics and subtropics in fresh or brackish water swamps, lakes, and ditches and along canal margins (Adams and Tomlinson 1979). This species is frequently found inland from coastal regions, sometimes associated with pines and palms or other glycophytes.

One of the primary factors determining the growth and distribution of plants in salt marsh habitats is the level of soil salinity (Jefferies *et al.* 1979). Other factors include both intraspecific and interspecific competition, especially when species may be only facultative halophytes. Barbour (1970) has questioned whether all halophytes are just facultative halophytes and has suggested that the ability to reproduce under "halophytic" conditions should be the ultimate criterion of salt tolerance. In halophytic pteridophytes, for sexual reproduction to be successful the gametophytic and sporophytic generation must succeed. Therefore, both generations should exhibit parallel tolerances to stress under soil salinity, assuming that the soil salinity conditions of the gametophytic and sporophytic habitats parallel one another. However, Ungar (1978) has reported that surface soils may have salinities from two to 100 times that of subsoils. Thus, the gametophytic stage may be critical if a species is to successfully inhabit a saline environment.

In the New World, collections have been made of both *Acrostichum* species. Gametophytes originating from spores of these plants as well as from the fresh water aquatic, *Ceratopteris thalictroides* (L.) Tod., have been grown under a variety of salinity conditions to test the hypothesis that tolerance to salinity by the gametophytic generation will parallel the habitat conditions in which the sporophytes occur.

MATERIALS AND METHODS

Spores utilized in this study were collected from the following locations: *A. aureum:* culture 190, Jamaica, Westmoreland Parish, 0.25 mile east of Negril on road to Savana la Mar. Plants occur densely in a large population in a lowland coastal swamp which is periodically inundated by tides; culture 193, Panama (Canal Zone), very large population of over 3000 individuals in mangrove swamp about 0.2 mile from road to Colon on road to Coco Solo; culture 150, Florida, Dade Co., 30.5 miles southwest of entrance of Everglades National Park, at road to Westlake, growing in Rhizophora-swamp; *A. danaeifolium:* culture 204, Florida, open marsh with about 40 individuals, Collier Co., 0.7 mile south of state route 92 on U.S. Highway 41, about 3.5 miles inland from coast; *Ceratopteris thalictroides:* culture 174, Guyana, two miles east of Georgetown on main coastal public road, in wet marsh with *Nymphaea* next to gasoline station; culture Hawaii, taro patches, Hawaiian Islands, inundated weekly with fresh water. Adscript numbers and letters designate spore progenies from different individual sporophytes collected at each location.

Spores were sown and gametophytes grown on inorganic nutrient medium solidified with 1% agar (see Klekowski 1969, for composition) in 100 x 15mm petri dishes under continuous illumination from fluorescent and incandescent lamps at about 23°C. Nutrient media were supplemented with NaCl prior to the addition of agar, yielding concentrations of 0.5 to 3.0%. NaCl concentration was ascertained by conductivity measurements (from 0.8mmhos/cm at 25°C in control to 44mmhos/cm in 3.0% NaCl.) Conductivity was measured with a Radiometer CDM2 conductivity meter. Data for 190-D/193-B (Table 4) represent gametophytes of both progenies transferred on to single petri dishes.

TABLE 1

Percent spore germination in *Acrostichum* (15 days following sowing) and *Ceratopteris* (12 days following sowing) at salinity regimes varying from 0.0% to 3.0% NaCl. (Sample size = 100).

Species and Culture Number

	A. aureum			*A. danaeifolium*		*C. thalictroides*		
NaCl (%)	**190-D**	**193-B**	**193-K**	**204-2**	**204-8**	**174-A**	**Hawaii-A**	**Hawaii-B**
0.0	69(100*)	66(96)	76(100)	99	89(100)	86(96)	95(100)	90(100)
0.5	55(80)	69(100)	61(80)	99	67(75)	90(100)	50(52)	20(22)
1.0	51(74)	60(87)	47(62)	92	72(81)	85(94)	30(32)	0
1.25	19(28)	44(64)	54(71)	72	54(61)	84(93)	0	0
1.5	11(16)	43(62)	56(74)	24	21(24)	19(21)	0	0
1.75	3(4)	19(28)	17(22)	28	8(9)	11(12)	0	0
2.0	1(1.4)	8(12)	17(22)	18	1(1)	2(2.2)	0	0
2.25	2(3)	2(3)	5(7)	3	0	0	0	0
2.5	0	1(1.4)	4(5)	0	0	0	0	0
2.75	0	0	1(1.3)	0	0	0	0	0
3.0	0	1(1.4)	1(1.3)	0	0	0	0	0

*Values given in () are adjusted to 100% to adjust for variation in intersporophytic spore viability.

RESULTS

Spore germination in all three species initially occurred five to six days following sowing and was highest in the control and the lowest NaCl concentration. There was a differential response of spore progenies from individual sporophytes of each species to each of the culture regimes. In *Acrostichum*, germination rates differ slightly at the higher NaCl concentrations (Table 1). Spore progenies of both species exhibited consistent but decreasing levels of germination up to 1.25% NaCl. At salinities above 1.25 and 1.5%, respectively, in *A. danaeifolium* and *A. aureum*, there are significant decreases in germination. At NaCl concentrations between 2.25 and 3.0%, spore progenies of *A. aureum* exhibited low levels of germination, whereas in the other species germination did not occur. There are indications of bimodality (stress pulses) in three of the five dose response curves (193-K, 204-2, 204-8 at salinities of 1.25-1.5%, 1.75%, and 1.0% respectively). If this pattern is real it could result from the operation of two physiological processes whose reaction optima occur at different levels of salinity.

In *Ceratopteris*, germination was greatly inhibited in Hawaiian spore progenies at all NaCl concentrations and did not occur above 1.0%. The Guyana sample is of potential interest, however, as germination responses were similar to those of *A. danaeifolium*.

The fraction of gametophytes to attain two-dimensional growth within 15 days of sowing was determined in order to evaluate the effect of salinity on developmental rates (Table 2). There is no appreciable difference in the two species of *Acrostichum* at the lower salinities with almost all gametophytes reaching the two-dimensional stage. The reaction curves for developmental rate versus salinity are markedly bimodal for the spore progenies of *A. aureum* tested. Developmental rates decreased synchronously for all four samples from both species between 1.0 and 1.25% NaCl. The second range of salinities (1.25 to 1.75%) which resulted in rapid gametophyte development for *A. aureum* produced reduced development in culture 204-2 and severely inhibited development at 1.75% NaCl in culture 204-8 of *A. danaeifolium*. The phenomenon responsible for the higher developmental rate in higher salinities for *A. aureum* appears to extend this species' maximum salinity tolerance beyond that of *A. danaeifolium*. However, it was not determined what proportion of 15 day old one-dimensional gametophytes later attained the two-dimensional stage under hypersalinity conditions.

TABLE 2

Mean percent attainment of two-dimensional morphology in gametophytes of *Acrostichum* grown under varying salinity regimes 15 days following sowing.

	Species and Culture Number			
	A. aureum		*A. danaeifolium*	
NaCl (%)	**193-B**	**193-K**	**204-2**	**204-8**
0.0	100	97	100	100
0.5	100	96	100	100
1.0	100	97	97	98
1.25	90	84	85	93
1.5	97	90	80	87
1.75	97	86	83	14
2.0	75	54	0	0
2.25	0	0	0	0

Sample size variable and dependent upon number of available gametophytes: 30-45 in 0.0% to 1.75%; (1)10-29 in 2.00%; 11-14 in 2.25%; less than 5 in remaining regimes.

TABLE 3
Maximum and (mean) gametophyte size (in sq. mm) attained in cultures of *Acrostichum* at salinity regimes varying from 0.0% to 2.25%. Sample size = 10.

	Species and Culture Number				
	A. aureum			*A. danaeifolium*	
NaCl (%)	190-D*	193-B*	193-K**	204-2***	204-8***
0.0	2.8(1.4)	10.9(3.8)	4.1(2.4)	11.8(5.1)	6.3(4.3)
0.5	1.9(1.0)	5.9(2.8)	13.9(2.0)	6.0(3.1)	8.5(3.2)
1.0	2.0(0.9)	4.0(1.8)	4.5(2.0)	4.0(2.1)	2.8(1.2)
1.25	0.4(0.3)	2.5(1.2)	1.8(0.8)	2.4(1.2)	1.9(0.9)
1.5	1.1(0.6)	1.2(0.7)	0.4(0.2)	0.7(0.4)	0.8(0.4)
1.75	———	0.6(0.4)	0.4(0.4)	———	0.09(0.08)
2.0	———	0.1(0.1)	0.2(0.1)	———	———
2.25	———	0.2(0.1)	0.09(0.07)	———	———

* sampled 19 days following sowing.
** sampled 18 days following sowing.
*** sampled 21 days following sowing.

TABLE 4
Mean percent non-chlorotic tissue in gametophytes of *A. aureum* grown 70 days in nutrient control medium and then transferred to variable salinity regimes.

	150-B: Days from transfer			190-D/193-B: Days from transfer		
NaCl (%)	3	14	21	4	7	15
0.0	99	91	91	100	100	100
1.0	100	99	99	100	100	100
1.25	94	86	94	100	99	92
1.75	91	80	83	97	91	69
2.0	71	74	75	97	90	72
2.25	73	62	58	87	74	69
2.5	40	27	0	76	81	39
2.75	45	31	0	38	25	13
3.0	40	30	0	32	23	0

Maximum and mean gametophyte area was measured 18 to 21 days following sowing to evaluate the effect of salinity on growth rates (Table 3). The growth rate of *A. danaeifolium* appears to be greatest in the 0% NaCl controls and then decreases linearly with increasing levels of salinity. Low NaCl concentrations (0.5 and 1.0%) reduced growth rates of *A. danaeifolium* to a greater extent than those of *A. aureum*. For example, reduction of mean gametophyte size (compared to 0.0% NaCl) of *A. aureum* progenies varies from 16.7 to 52.7%, whereas reduction in size of *A. danaeifolium* progenies is 58.9 to 72.1%.

Salt stress and development of chlorotic tissue was measured in gametophytes of *A. aureum* (Table 4). These gametophytes were grown to maturity for 70 days on control media and then transferred to various salinity regimes. In culture 150B, below 2.0% NaCl there is no apparent difference over time but only with salinity concentration. The effect appears to be initial and then persistent. There is some indication of a stress pulse at 1.25 to 2.0% NaCl with gametophytic tissue recovery after 21 days. Above 2.25%, the salinity effects are progressive over time. In culture 190/193, effects appear to be absent below 1.25% NaCl. At levels of 1.25% and above, there is a progressive increase in chlorotic gametophytic tissue with both time and NaCl concentration.

DISCUSSION

The tolerance to salinity of gametophytes of both species of *Acrostichum* appears to be significantly greater than two of the three progenies of the glycophyte *Ceratopteris thalictroides*. Warne and Hickok (pers. comm.) have also studied NaCl effects on spore progenies of *C. thalictroides*. They analyzed spore germination and gametophyte survival of progenies from ten sporophytes from various parts of the World. These progenies exhibit a wide range of tolerance to NaCl. At 0.8% NaCl, spore germination in six of the ten progenies was reduced by 4.0 to 15.9 (mean = 7.53%) compared to the controls (0.0% NaCl). In the remaining four progenies, germination was reduced 48.8 to 97.3 (mean = 77.7)%. Twenty-one days following sowing, gametophytes of the former group were 2.5 to 10% the size of control gametophytes, whereas gametophytes of the latter group failed to survive. These results indicate that some populations of *Ceratopteris* may have salinity tolerance approaching that exhibited by *A. danaeifolium*. Recently, Petersen (1985) reported that spores of *A. danaeifolium* were capable of germinating in up to 2.5% NaCl whereas spores of the glycophytic species *Osmunda* spp. and *Onoclea sensibilis* ceased germination at 0.6% NaCl.

In *Acrostichum*, there is a wide range of tolerance to salinity, although *A. aureum* consistently shows a slightly greater tolerance at slightly higher NaCl levels. In spore germination, two of the three progeny samples of *A. aureum* show 50% germination at NaCl concentrations of 1.5 and 1.75%. In *A. danaeifolium*, 50% germination occurs between 1.25 and 1.5%. Similarly, the critical salinity level for attainment of two-dimensional morphology is 1.75 to 2.0% NaCl in *A. aureum* and 1.5 to 1.75% in *A. danaeifolium*. In addition, some progenies of mature gametophytes of *A. aureum* can tolerate prolonged exposure to salinities of 2.0 to 2.25%. These results suggest that the critical soil salinity which will limit gametophyte survival of *A. danaeifolium* will be about 1.5% and of some plants of *A. aureum*, about 1.75 to 2.0%. However, due to the extremely large number of spores produced by individual sporophytes of these species, even very low percentages of survival at higher salinities could result in millions of successful gametophytes.

Germination and growth in *Acrostichum* parallels that of other mangrove species which have been studied and have optimal growth between 0.6 and 1.5% NaCl (Connor 1969; Pannier 1959; Patil 1964).

The spore germination and gametophyte growth patterns of *A. danaeifolium* are similar to those described for semi-halophytes by Waisel (1972). Semi-halophytes show a slow decrease in growth at initial stages of salinity increase, followed by a steady decrease with increasing salinity. On the other hand, in *A. aureum*, the stress-pulse growth phases followed by a steady decrease in growth with increasing salinities is similar to the pattern of true halophytes. These studies indicate, therefore, that species of *Acrostichum* can be considered to be semi-halophytes or halophytes and that the salinity tolerance by the gametophyte generation is an integral part of their life-history.

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