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The effects of aluminum concentration on growth responses in six Spartina alterniflora genotypes

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THE EFFECTS OF ALUMINUM CONCENTRATION ON GROWTH RESPONSES IN SIX *SPARTINA ALTERNIFLORA* GENOTYPES

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ABSTRACT

Elevated soluble aluminum concentrations can adversely affect plant growth. During a drought, wetland soils may experience higher than normal soluble aluminum due to the oxidation of metal sulfides and resulting decreases in pH, which mobilizes metallic cations. Louisiana coastal salt marshes were subject to a record-setting drought in the winter and spring of 2000 which was coincident with the die-off of large expanses of salt marsh, termed "brown marsh". *Spartina alterniflora* was the primary plant species affected. However, because some individuals within large areas of die-off survived the brown marsh event, they may have been the more resistant genotypes. To determine if genotypic resistance to aluminum existed, six genotypes of the common salt-marsh cord-grass *Spartina alterniflora,* five surviving genotypes, and a commercial variety (Vermillion), were dosed with aluminum chloride ($AlCl_3$) at concentrations ranging from 0.2 mM to 10.8 mM. No death was observed in any of the genotypes at aluminum concentrations as high as 10.8 mM, although growth rates decreased to near zero. The results of this study indicate that, as a species, the resistance of *Spartina alterniflora* to aluminum may surpass the threshold of any plant species studied to date. All genotypes in the experiment were found to tolerate extremely high concentrations of aluminum, although declines in stem elongation rate and cumulative stem height were evident in all Al treatments. I estimated the differential aluminum tolerance by using the first significant decrease in growth rate when the genotype x concentration effect was significant. The first significant decrease approach had the best resolution for determining genotype variability when used with the stem elongation data. Although insufficient evidence exists to determine if aluminum toxicity caused the brown marsh event in Louisiana, based on the results of this thesis, the aluminum concentrations would have had to reach extremely high levels to have been the sole cause of the brown marsh dieback.

CHAPTER 1

INTRODUCTION

Aluminum, a very common and abundant element in many soils, is normally not toxic in salt marsh soils due to chemical equilibria maintained by pH conditions buffered near neutrality and reduced redox potential (Gambrell 1994). Soils that are normally water saturated are under reduced conditions, meaning dissolved oxygen is depleted and the reduced form of many elements including iron, manganese and sulfur are present. When normally flooded and reduced soils are allowed to dry out, such as in rice cultivation, oxidized-acidic conditions may develop and high concentrations of soluble metals may develop (IRRI 1978). Under acidic conditions, plants may become stressed by a high availability of potentially toxic metals (Prasittikt and Gambrell 1989).

Coastal salt marsh soils are potential acid sulfate soils. When seawater floods reduced coastal marsh soils, sulfate is biochemically reduced to sulfide, which reacts with Fe(II). Pyrite (FeS_2) is one of the iron sulfide minerals that can form. Pyrite formation in wetland soils has been reviewed by Prasittikhet and Gambrell (1989). Pyrite is stable under reduced conditions. Oxidation of soils through lowering of the water table causes pyrite to oxidize to sulfuric acid (H_2SO_4). The formation of acid decreases the pH and causes Al^{3+}, Fe^{2+}, and other metals, to become mobile in the soil solution. In acid sulfate soils, sulfuric acid formation can reduce the pH to 4 or less (Prasittikt and Gambrell 1989, McKee and McKevlin 1993).

The dominant plant species in Louisiana coastal salt marshes, *Spartina alterniflora* Loisel. (Poaceae) recently (2000) underwent a massive die-off (termed “brown marsh”) (McKee *et al.* 2004). The brown marsh event occurred during a drought, but the exact cause of the die-off is undetermined. One potential cause of the event could be oxidation of soil metal sulfides

resulting in increased acidity and metal toxicity (McKee *et al.* 2004). Aluminum toxicity due to acid sulfate soils negatively affects rice (IRRI 1978) production and may similarly impact the growth of marsh vegetation.

Species have genotypic differences in growth response to aluminum toxicity (Macedo *et al.*1997). Yamamoto *et al*. (1996), and Ishikawa and Wagatsuma (1998) found that some ecotypes can be more tolerant to Al than others. If *Spartina alterniflora* shows such ecotypic differences in Al toxicity, this may explain differential survival during the brown marsh event.

Water stress may exacerbate the impacts of Al toxicity. Schier and McQuattie (2000), investigating the effect of water stress on Al toxicity, theorized that an increase in water stress due to drought would enhance Al toxicity.

The exact cause of the brown marsh phenomenon is currently under investigation and unexplained. However, aluminum toxicity is one potential cause of brown marsh. The objectives of this research were to: (a) quantify the Al concentration that exhibits a toxic effect on *Spartina alterniflora*, (b) determine variability in resistance of five *Spartina alterniflora* genotypes that survived brown marsh conditions and one commercially available cultivar, and (c) determine which growth parameters, if any, distinguish genotype resistance. The objectives were accomplished by growing the *Spartina alterniflora* genotypes in a controlled hydroponic environment while the experimental units received increasing concentrations of aluminum.

CHAPTER 2

BACKGROUND

This literature review is about soil-plant interactions contributing to plant aluminum toxicity. Interest in this topic is derived from the recent brown marsh event in Louisiana where extended drainage and soil oxidation may have contributed to pyrite oxidation and subsequently a sufficiently low pH to mobilize aluminum and perhaps some other elements to plant-toxic levels. Aluminum toxicity is just one of several possible contributors to the brown marsh event. This review focuses on soil conditions and plant responses that may enhance the toxicity of aluminum**.** The methodology of metal toxicity screening of cultivars was reviewed in preparation for the experimental design (Reid *et al.* 1971, Howeler and Cadavid 1976, Schier 1996, Macedo *et al.* 1997, Wagatsuma and Ezoe 1985, Rahman *et al.* l998, Lidon and Barreiro 1998, Sun and Wu 1998, Lux and Cumming 1999, Schier and McQuattie 2000).

1 Soil Chemistry

Aluminum is the most common metal in the lithosphere and soils. (Delhaize and Ryan 1995). Although aluminum is abundant in the mineral fraction of soils, the concentrations of plant-available metals in the soil solution usually remain in trace quantities, but can increase to toxic levels depending on soil physio-chemical conditions. Aluminum is thought to be the largest contributor to upland soil acidity (Van Breeman and Moorman 1978). Soil fractions affecting the toxicity of metallic ions vary with the chemical environment. Mineral clay particles weather over time and release Al into the soil as mobile or potentially mobile forms. Metals enter the coastal marsh from mineral matter transported from the watershed.

When acidified rainwater caused dissolved Al concentration to increase to 33 μM in stream-drained catchments (Anderson and Seip 1999), it was theorized that the acid conditions

increased salinity and mobilized aluminum in mineral soil drainage. Anderson and Seip (1999) found that the cation exchange capacity (CEC) of the organic soil fraction to be inversely related to dissolved inorganic Al^{3+} concentration, while the clay minerals (gibbsite, jurbanite, kaolinite/halloysite, and imogolite) had no noticeable effect on soluble Al^{3+}concentration.

a. Soil Minerals. Clay carries a pH-dependant, usually negative, charge on the surface of the particles. The cation exchange capacity depends on the amount and type of organic matter and clay, and also upon the pH (Brady and Weil 1996). Due to the charge on the surfaces of the clay minerals, metal ions in the bulk soil solution are in equilibrium with exchangeable ions bound to clay minerals. The charge on clay minerals is variable and influences the exchangeable ions in solution. The surfaces of clay minerals such as gibbsite $Al(OH)_3$, hematite $Fe(OH)_3$ and geothite [FeOOH] express negative or positive charge depending on the pH of the soil solution. A low pH will cause protenation and a positive charge will form on the surface of hematite and goethite, $\{ –Fe\text{-}OH + H^+ \leftrightarrow FeOH_2^+\}$. A high pH causes deprotenation and negative surface charge $\{–FeOH + OH^- \leftrightarrow –Fe\text{-}O^- + H_2O\}$. In gibbsite, the clay surfaces can likewise have variable charges$\{ –Al\text{-}OH + H^+ \leftrightarrow \text{--}AlOH^+_2$ and $–Al\text{-}OH + OH^- \leftrightarrow –Al\text{-}O^- + H^+\}$ (Foth and Ellis 1996). When soils with high metal holding capacity undergo a substantial pH decrease, then metals can be mobilized and contribute to plant toxicity. Soils with low CEC usually have low metals content.

b. Soil Organic Matter. Organic matter (OM) composes much of the volume of soil in many coastal salt marshes. In flooded soils, the organic component may be the most important feature of Al availability. Humic substances formed from incomplete decomposition of plants, animals, and microbes are responsible for: (A) providing an energy substrate for microbial activity, (B) strong binding of metals by a process known as chelation, and hence removal of toxic metals

from solution, and, sometimes in an opposing process, and (C) metals complexed with soluble-low-molecular-weight-organic-matter, which can result in increased soluability and mobility of metals (Manahan 1994, p 81).

The variable negative charge of OM influences the CEC of a soil and is pH dependent. The relatively high CEC of soil organic matter, coupled with the chelation capacity and abundance of organic matter in many coastal marsh soils, is very effective in immobilizing metals. Chelation is a very strong bonding mechanism, and it takes drastic soil changes like the oxidation of OM or a low pH to release this chelated form. Aluminum forms one of the strongest metal bonds with humic substances (Manahan 1994). Particulate OM (POM) is important to metal solution chemistry because, like clay, the cation exchange and metal chelation occurs in the insoluble organic component. Particulate matter is not the only organic component of the soil influencing metal toxicity.

Dissolved organic matter (DOM) may also alter the metal toxicity by chelating metallic ions into a mobile, charge-netural form of metals (Manahan 1994). Also, DOM such as fluvic acid may alter the toxicity of Al by prohibiting the root from maintaining an oxidized rhizosphere (Van Breeman and Moorman 1978 p791). Anaerobic microbes are less effective than aerobic microbes in metabolizing organic carbon sources so that more by-products of OM decomposition remain. Under reducing conditions, humic material is more abundant and more structurally complex than under oxidizing conditions due to low energy-electron acceptors, and this results in better metal retention capacity (Gambrell *et al.* 1991).

c. Soil pH. The concentration of free hydrogen ions in solution causes changes in the speciation of Al and pH that affects the solubility of toxic metal ions. Aluminum ions in hydrated forms contribute to the acidity of the soil solution as follows: $Al(H_2O)_6^{3+} \leftrightarrow Al(H_2O)_5OH^{2+} + H^+$

Submergence of oxidized acid soils with adequate iron causes the pH of acid soils to increase. Buffers in submerged soils include the products of anaerobic microbial respiration. Iron and manganese hydroxides buffer the soil solution by shifting the pH toward neutrality (DeLaune *et al.* 1976). In many soils, as pH drops to 5.0 and below, the soluble levels of aluminum increase to plant-toxic levels (Foy 1974). Delhaize and Ryan (1995) reviewed Al speciation with respect to pH. Monomer aluminum (Al^{3+}) is found under acidic conditions (pH <5). An increase in pH will cause $Al(OH)^{2+}$ to form. Further increase in pH will cause $Al(OH)_2^{+}$. The Al mineral gibbsite $Al(OH)_3$ forms at neutrality. Alkaline conditions cause aluminate ($Al(OH)_4^{+}$) to form. In a review by Kinraid (1991) all of the Al species were found to be toxic. Waggatsuma and Ezoe (1985) investigated the effect of varying pH on Al uptake by plants. In nutrient culture, monomeric Al^{3+} exists at pH 4.1. Increasing the soil solution pH to 4.7 releases free aluminum hydroxide and precipitated or polymerized Al ions. Less Al uptake was associated with the monomer form of aluminum (found at lower pH). Increased Al toxicity may be associated with polymer Al (found at higher pH).

2 Toxic Effect.

Both Fe and Al toxicity can cause leaf bronzing or tissue necrosis. Bronzing occurs when shoots loose their green color (Van Breeman and Moorman 1978), although there have been experiments where leaves do not turn color but the plants stop growing. Fe and Al may also cause micro-nutritional disorders. The known physiological toxic response of plants to Al are: (A) interference in cell division, (B) P is fixed to less available forms, (C) a decline in root respiration, (D) the disturbed enzymatic deposition of polysaccharides in the cell wall, (E) a rigidity of cell walls, and (F) disruption of Ca, Mg, P, and K uptake, transport, and

metabolization (Foy *et al.* 1978). In strongly acid sub-soils, Al toxicity results in a shallow depth of rooting, loss of drought tolerance and lower accessibility to subsoil nutrients (Foy *et al.* 1978).

a. Symptoms. The toxic effect of Al causes symptoms resembling P or Ca deficiency. P deficiency may cause stunted plants with small dark-green leaves, purple stems, leaves, and leaf veins. Ca deficiency causes young leaves to curl or roll, and the petiole to collapse (Foy *et al.* 1978). Al toxicity affects root development and decreases root length (Reid *et al.* 1971). Root tips and lateral roots become thick and brown from Al toxicity. Lateral roots are short and fragile with few fine branches, thus the nutrient and water availability of the plant is affected (Foy *et al.* 1978).

b. Target Region. Kochian (1995) conducted a review of research on Al phytotoxicity. There appears to be an agreement among researchers that the root apex is the primary target region of metal toxicity. Toxic effects are noticed after as little as 1 to 2 hours of Al exposure. The initial Al toxic response is the suppression of root elongation.

c. Synergistic Effect. Yamamoto *et al.* (1996) reported peroxidation of plasma membrane lipid by Al in conjunction with Fe(II). Previously, Gutterridge *et al.* (1985) found that Fe(II) at low pH could cause peroxidation of lipids at a faster rate when Al was added due to Al ions enhancing destruction of the membrane structure.

Delhaize and Ryan (1995) revealed that 0.1 mM $FeSO_4$ alone did not affect cell viability; although, only a low concentration is sufficient to cause cell death with Al present. Toxic effects were noticed 10 days after an 18 hour treatment of cells with 0.12 mM $AlCl_3$ (Delhaize and Ryan 1995). This study indicated that Fe and Al have synergistic effects and was consistent with the findings of Ono *et al.* (1995).

3 Plant Adaptations

Plants growing in flooded soils suppress toxic environmental conditions by root exudates. Specialized aerynchma cells transport oxygen to roots and into the root zone. Ferric oxide plaques form on the roots due to FeS reacting with oxygen and precipitating FeOH onto the root (Mendelsssohn and Postek 1982). Plaques may protect the plant from further metal toxicity by blocking root uptake of other metal cations (Van Breeman and Moorman 1978). The effect of root tissue CEC and soil modification by root exudates on Al uptake is under debate. Isikawa and Wagatsuma (1996) found evidence that contradicts the findings of Watasuma and Ezoe (1985) regarding an ability of plants to adjust the CEC and therefore the uptake of metals by the roots.

Plants may alter the pH of their soil solution to maintain electrochemical gradients in roots. Foy (1978) reviewed the mechanisms of aluminum tolerance. Some plants modify the soil pH by root exudates. Aluminum resistant cultivars have mechanisms inducing a higher pH which causes aluminum to decrease in solubility. The pH of the growth media is variable through anion-cation selective uptake. Aluminum sensitive cultivars decrease the pH of growth media. Selective uptake of NH_4^+ causes a decrease in pH. The pH change may also be attributed to increased CO_2, or the release of H^+ ions and the excretion of protons. The fitness and nutrition of the roots may have a strong influence on pH change. When the plants are no longer able to deal with toxic environmental conditions outside the root, then internal tolerance strategies become important for plant fitness.

4 Tolerance

Foy (1978) reported on processes found that plants undergo to tolerate toxic Al concentrations: (A) roots of tolerant cultivars do not contain as much Al as sensitive cultivars (B)

Al is excluded from shoots by trapping Al in roots, (C) concentrating Al in plant shoot allows the leaves to have lower Al levels, and (D) concentrating Al in older leaves and in the plasmalemma of meristem formed in a way that blocks Al from uptake.

The strategies needed to increase aluminum tolerance may be similar to what plants do for iron. A study by Alberts *et al.* (1990) using *Spartina alterniflora* showed that both Al and Fe are blocked from uptake into roots. The plants were found to have a low concentration factor for these elements suggesting that active uptake does not occur. The researchers discovered little translocation of both Al and Fe from roots to stems and leaves. Further, there was little difference in the stem and leaf concentrations.

5 Screening Methods

The aluminum toxicity of genotypes within a species was evaluated by de Macedo *et al.* (1997). To rank Al toxicity for genotypes of rice, they suggested that multiple measurements are necessary to determine the relative Al toxicity among genotypes of the same species. Root morphology was a better indicator of toxic response than root length or weight. Stem measurements could be variable due to restricted or promoted root development. Using a necrosis criterion may be the only reliable method to gauge Al toxicity in long-term experiments at high concentrations of Al. At low concentrations and short intervals of exposure, plants would be categorized best by weight parameters, not length parameters. The morphology of the roots was always an indication of Al toxicity.

Ishikawa and Wagatsuma (1998) studied the effect of $AlCl_3$ on root tip cells after brief exposure of seedling roots to determine the plasma membrane permeability of root tip cells. Their results suggest that a 0.5 hours exposure to the roots of the whole plant, or 10-minute exposure of protoplast, may be all that is needed to determine if a plant has reached a tolerance

threshold for aluminum. The researchers suggest that a similar technique may be used to determine tolerance variance in cultivars of a single species and claim to have unpublished results supporting this.

Yamamoto *et al.* (1996) found that Al ions at pH 5 were a major growth limiting factor for cultured tobacco cells. Aluminum inhibits root growth within 1 to 2 hours, and cells in logarithmic phase of growth are Al sensitive, while cells in the stationary phase are no longer Al sensitive. Thus only actively dividing cells are sensitive to Al toxicity.

6 Toxicity Threshold

Schier (1996) determined if there were differences in the Al-threshold toxicity of new and one year old red spruce seedlings. The needle dry weight and stem dry weight toxicity threshold was greater for the younger age groups (0.4 mM Al for 1 year old spruce and 0.8 mM Al for young seedlings). An aluminum concentration of 0.4 mM Al significantly decreased plant height causing the toxicity threshold based on plant height.

Lidon and Barreiro (1998) developed a dose response curve for maize in order to determine at which concentration a threshold toxicity occurred. The researchers discovered a threshold level of 13 $\mu g \cdot g^{-1}$ for maize. Plants were dosed with 0 to 3.0 mM Al at pH 4. The researchers compared their dose-response curve with that of others and concluded that the toxic effect began at tissue concentration of 13 $\mu g \cdot g^{-1}$(dry weight). The threshold for a plant effect appears to occur above 0.3 mM Al. Both root and shoot fresh weight and dry weight increased when the Al concentration was increased from 0 to 3 mM Al. An Al dose of 0.9 mM Al caused a decline in plant weight.

Thorton's Critical toxicity level is the concentration of toxic metal ion that caused experimental treatments to decrease below 20% of control (Lux and Cumming 1999). Lux and

Cumming (1999) determined the Thorton's critical toxicity level for tulip poplar seedlings. The range where approximately 70% of damage occurred was between 0 and 0.2 mM Al. The critical toxicity level for tulip-poplar was determined to be 0.190 mM Al (root tissue toxic concentration was 0.512 mM Al).

Sun and Wu (1998) determined the toxicity threshold concentration of water spinach. The plants were grown in cultures ranging 0 to 1.7 mM Al. Plants began to show symptoms of toxicity at 0.7 mM Al. The toxicity threshold was stated to be 1.7 mM Al.

Barcelo and Porschenrieder (2002) noted three Al dose response models related to Al toxicity over short intervals or low concentrations. The decreasing curve was related to a toxicity threshold, another represents a stimulation effect at the lower dose or shorter time interval, and the remaining curve shows a lag effect. Respectively these three responses are called the "threshold for toxicity", "hormesis model", and "threshold for tolerance".

CHAPTER 3

MATERIALS AND METHODS

The experiment was designed to subject cultivars of *Spartina alterniflora* to concentrations of Al high enough to cause a toxic response to the plant. The goal of the experiment was to compare six genotypes which survived throughout the brown marsh event (the brown marsh event was a massive die-off of marsh grasses which occurred during a prolonged drought in Louisiana (McKee *et al.* 2004)).

The experimental design aimed to recreate the conditions that could result in metal toxicity in a wetland. The literature suggests that a pH<4 would be required to maintain the Al in the mobile form (Reid *et a*l. 1971, Howeler and Cadavid 1976, Schier 1996, Macedo *et al*. 1997, Wagatsuma and Ezoe 1985, Rahman *et al.* l998, Lidon and Barreiro 1998, Sun and Wu 1998, Lux and Cumming 1999, Schier and McQuattie 2000). The interpretations of results from the initial tests were that sulfuric acid was a more reliable method of adjusting and maintaining the pH than was hydrochloric acid. I reviewed the literature to determine the concentration range where toxicity thresholds had been determined for other species (Reid *et al*. 1971, Howeler and Cadavid 1976, Schier 1996, Macedo *et al.* 1997, Wagatsuma and Ezoe, 1985, Rahman *et al.* l998, Lidon and Barreiro 1998, Sun and Wu 1998, Lux and Cumming 1999, Schier and McQuattie 2000). After a four week acclimation period, data collection and Al concentration increases began. The Al concentrations for the treatment plants were increased every two weeks to create the following concentrations: 0, 0.2, 0.6, 1.8, 5.4, 10.8, 10.8 mM. The plants serving as controls received no Al. The aluminum concentration was increased to a higher level after every two weeks when the nutrient solutions were changed.

1 Samples. Specimens of *Spartina alterniflora* were obtained from the USDA-NRCS-Golden Meadow Plant Materials Center, Galliano, Louisiana. Five wild genotypes that survived the brown

marsh event were designated as: 11T, 6T, 3D, 7D, and 16D. One genotype was a commercially available cultivar 'Vermillion'. Vermillion is currently the only cultivar approved for government funded wetland restoration projects. A random numbers table was used to select specimens from stock plants, and then to allocate them to control and experimental treatments. I selected 48 specimens representing 8 of each of the six genotypes. Four replications of each genotype in both control and experimental groups were employed.

Care was taken to separate each of the specimens from their existing containers and to meticulously wash the roots. The wet weight of each plant was recorded. The specimens were transplanted into the prepared sand media and hydroponic solution. A four week acclimation period was provided before data collection began.

2 Aluminum Aluminum in the form of $AlCl_3$ was used to increase the concentration of aluminum every two weeks as follows: 0, 0.2, 0.6, 1.8, 5.4, 10.8 mM. An additional two week interval at 10.8 mM was employed to further increase the chance of identifying an effect at this concentration. Howeler and Cadavid (1976), Archambault et *al.* (1996), Ishikawa and Wagatsuma (1998) and Lidon and Barreiro (1998) used aluminum chloride ($AlCl_3*6H_2O$) for toxicity tests, and this form was therefore used in the present study. The pH was kept at or below 4 to maintain the dissolved, $Al^{3+}_{(aq)}$ form.

3 Apparatus. Two identical ebb-and-flow hydroponic tables were constructed to fit within a growth chamber. One control unit and one experimental unit supported 24 plants (48 total) within the flow table. Plants were potted into 1 gallon pots. Each flow table was connected to a 100 L sump. A 110 volt appliance timer was used to cycle two 12 volt-10 amp power transformers on or off every 15 minutes. Two 7570 liter per hour 12 volt-10 amp centrifugal submersible pumps were attached to the floor of each sump. The pump return and the wier-drain were placed on opposite ends

of the flow table. Twenty-four containers, lined with hardware cloth and filled with acid-washed sand and a transplanted sample were contained within each flow table. When the pump was operated, a solution would flood the table and flow around and into the plant containers. The water level was adjusted by a weir to be above the surface of the sand in the pots. The substrate volume within the pots was adjusted so that all of the containers were similarly inundated when flooded. The solution level was never allowed to rise above the rim of the container.

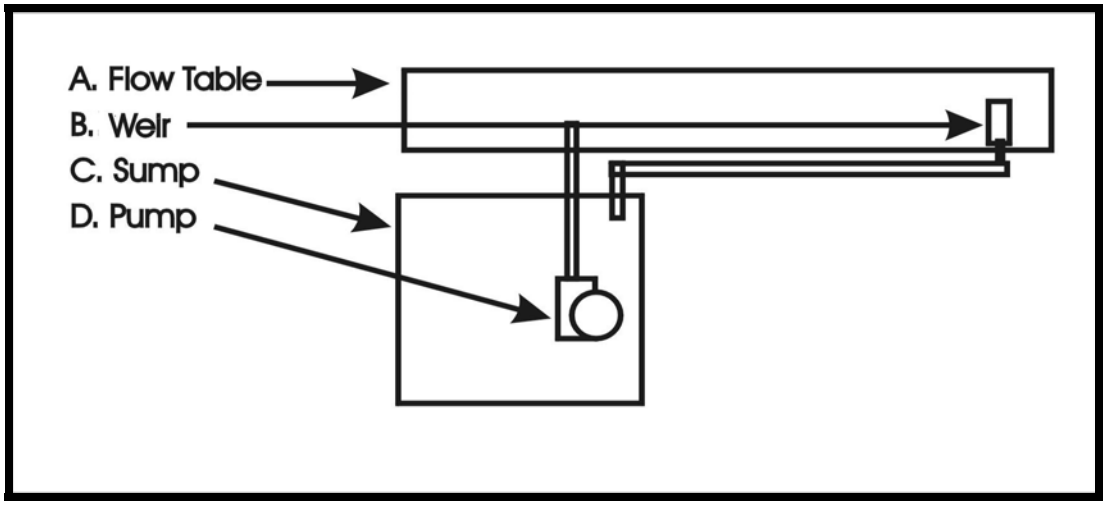


Figure 1. Schematic of the apparatus used in this experiment. The flow table (A) supported the plant containers. The weir (B) restricted the water height. The sump (C) held 100 L of water. The pump (D) was operated to flood the table.

4 Hydroponic Solution. A hydroponic nutrient solution was made by adding 50% dilute Hoagland's stock solution (Hoagland and Arnon 1950) to each 100 L of tap water. Before the Hoagland's stock solution was added, the water was prepared by the following additions: Instant-Ocean™ salt was added to create a salinity of 23 ppt. The pH was reduced to below 4 with 1N H_2SO_4 before any metal additions to prevent Al precipitation.

After 7 days, the evaporated water was replaced, the pH was measured, recorded and adjusted, the salinity measured, and N and P was added to replace lost nutrients. A reagent test kit (CHEMets®) was used to monitor N and P concentration to determine when additions were

necessary. The solution was replaced after 14 days and the Al concentration was increased in the experimental unit. Bi-weekly maintenance additions of 1N H_2SO_4 were required to maintain pH≤4.

5 Media. We used sand culture to be certain the Al would remain in solution and not bind to exchange sites. Sand was prepared by rinsing and leaching approximately 320 lbs of Play-Sand™ in a trash can fitted with a bottom drain and a filter. The sand was leached and rinsed for 12 hours using a garden hose and then drained. This procedure was repeated two times. Muratic acid (dilute HCl) was added until the sand was submerged. The acid was allowed to drain 24 hours later. A second 24 hour acid bath was followed by rinsing and draining thrice. After leaching with acid and washing, most visible traces of calcareous particles, silts, clay and organic material were removed. The remaining sand was well graded course sand sized particles.

6 Data Collection. The data were collected at two week intervals. This occurred the second week following nutrient solution replacement and dosage adjustments. Data were recorded on the last day of the second week. On that day, nutrient solutions were changed and Al concentration was increased. Stem elongation rate was determined by marking one low to medium height stem with lab tape and recording the stem height initially and after a 3 day interval. The stem height of each live stem within each container was measured in centimeters from the top of the container using a meter ruler. A 1cm diameter dowel was placed across the rim of the trade gallon container to determine the base for the ruler. This method only measured stems that were taller than the rim of the container. Live stems including stems shorter than the rim of the container were counted.

The stem elongation rate (cm/d), cumulative stem height or the total of all the stem heights for each container (cm), and relative growth rate (cm cm^{-1} day^{-1}) were computed from the raw data. The stem elongation rate (cm/d) was calculated for each container by first finding the difference in the stem height data and dividing by the time interval [(stem height $_{day\ 3}$ - stem height $_{day\ 1}$) / 3 days].

The mean and ±1 standard error was graphed as a percent of the control treatments.

The stem count and cumulative stem height data of each sample were combined by genotype and into four control and four experimental samples, and then their means and standard errors were graphically reproduced as both a percent of the control and as a relative growth rate. The relative growth rate (RGR) was determined by $RGR= (\ln X_2 - \ln X_1) / (t_2 - t_1)$ for the cumulative stem height and stem count, where X is the growth parameter and t is the time. The mean and ±1 standard error was graphed as a percent of the control treatments and as a relative growth rate.

The necrotic tissue was removed throughout the experiment and combined with the final biomass for final weighing. The root material was separated from the stem material on the last day. The separated biomass was put in an oven for 72 hrs at 80˚C. The material was stored in a cooler until final weighing.

7 Statistical Analysis. The statistical analysis was conducted using SAS's (version 8.0) Mixed Model with repeated measurement (SAS 1998). Least square means was used to compare between individual treatments when the interaction term was significant. A Saxton's macro for converting mean separation output to letter grouping in Proc Mixed was used (SAS 1998). The significance was reported at probability of 0.05 unless otherwise mentioned. The measured variables were converted to the percentage of the control treatment of each genotype.

CHAPTER 4

RESULTS

1 Stem Elongation Rate

The stem elongation rate, averaged over genotype, significantly decreased with increasing aluminum concentration ([Al]) (data expressed as percent of control) (Figure 2A, Table 1 – Concentration Effect). However, the effect of increasing [Al] on stem elongation was significantly different with genotype (Figure 3, Table 1 – Genotype x Concentration interaction). For example, the stem elongation rate of the Vermillion genotype significantly increased as [Al] increased from 0 to 1.8 mM (Figure 3). However, the stem elongation rate of the Vermillion genotype decreased from its peak, relative to controls, at [Al]'s greater than 1.8 mM. The stem elongation rate of the genotype Vermillion then decreased to almost zero relative to the control treatment. Although some of the genotypes (3D, Vermillion, 7D, and 6T) showed resistance to low [Al]'s, other genotypes, specifically 16D and 11T, exhibited decreased stem elongation even at relatively low [Al]'s (Figure 3 and Table 2). If one utilizes the [Al] at which the first significant decrease in stem elongation occurred as a measure of sensitivity to Al, the genotype 16D was the most sensitive genotype to increasing [Al], while genotypes 7D, 6T, and 3D were the least sensitive and Vermillion and 11T were intermediate (Table 2).

Table 1. The results of an analysis of variance type 3 test of the fixed effects of aluminum concentration on stem elongation rate.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
Genotype	5	5.1	0.53	0.7502
Concentration	6	73.8	55.14	<0.0001**
Genotype x Concentration	30	34	2.12	0.0177*

*Significantly different $p<0.05$
** Significantly different $p<0.01$

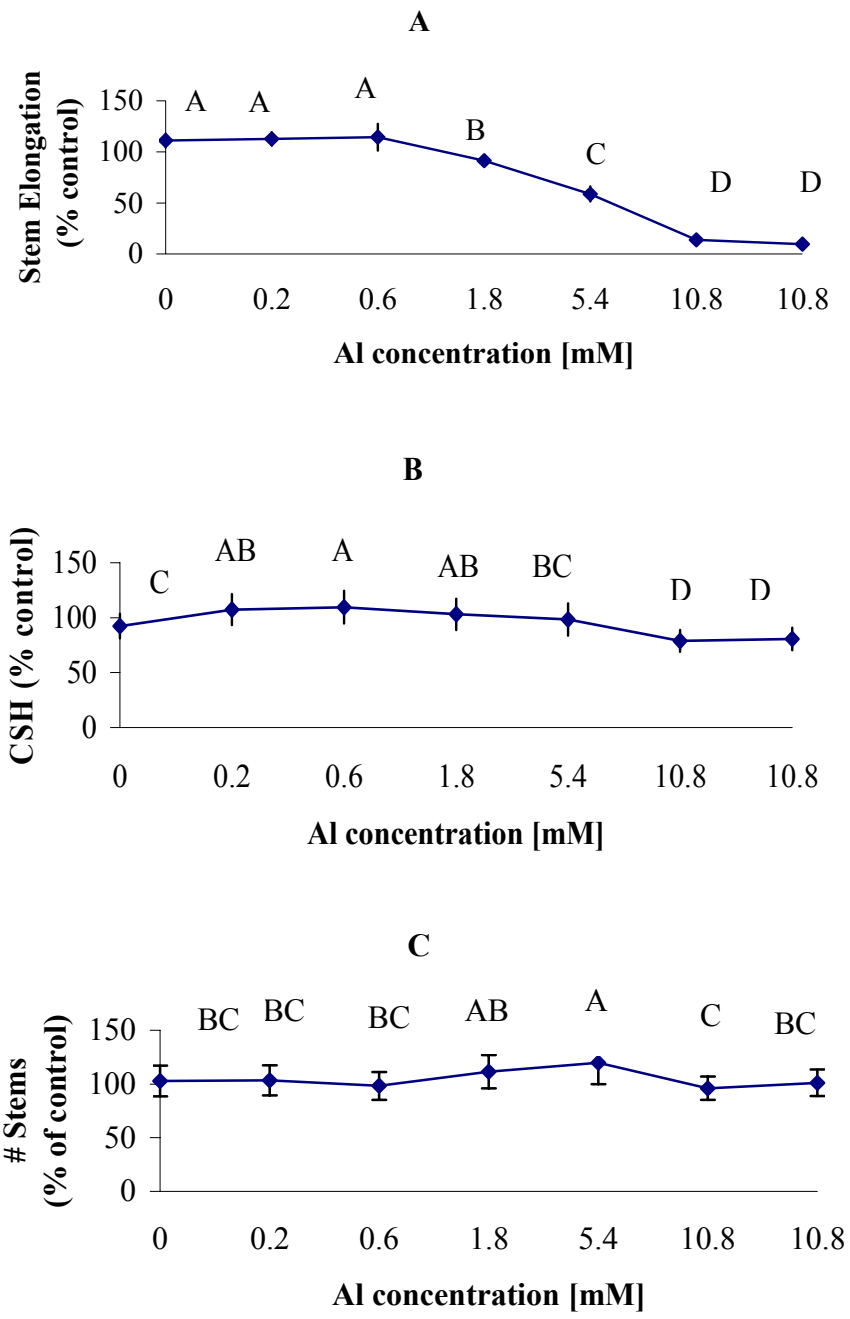
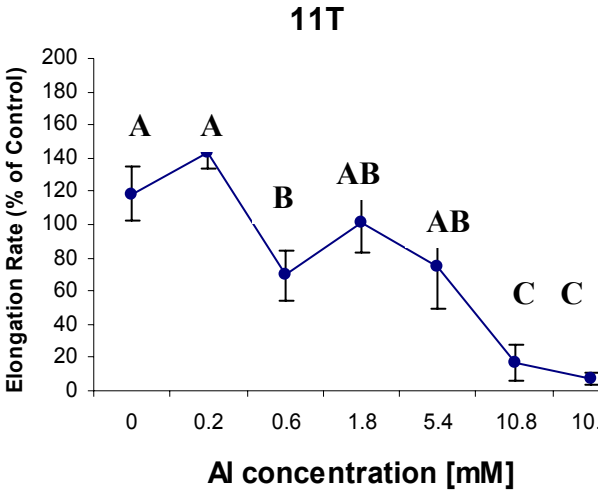
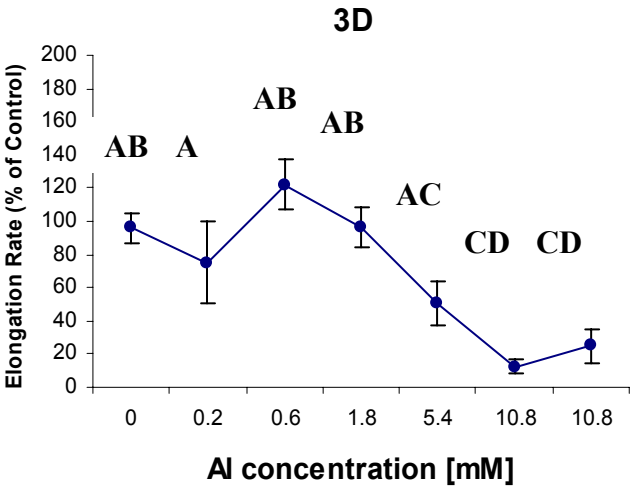
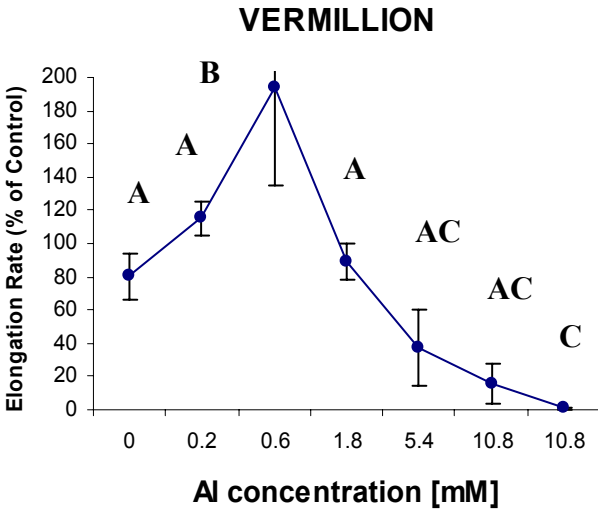


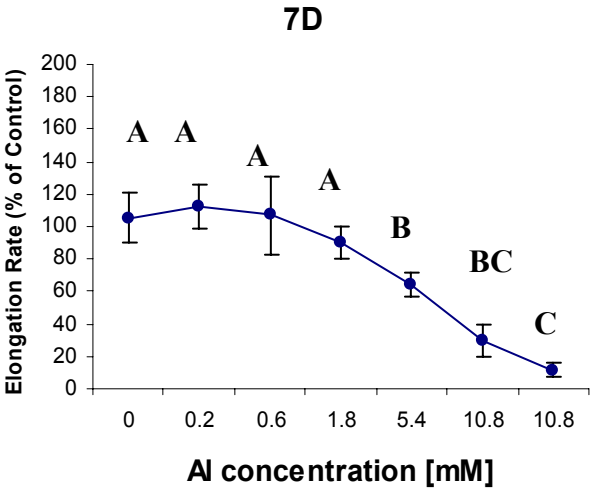
Figure 2. The main effect of aluminum concentration, averaged over genotype, on stem elongation (A), cumulative stem height (CSH) (B), and number of stems (# Stems) (C) (n=24). The error bars are ±1 standard error. The different letters indicate significantly different means ($p<0.05$).

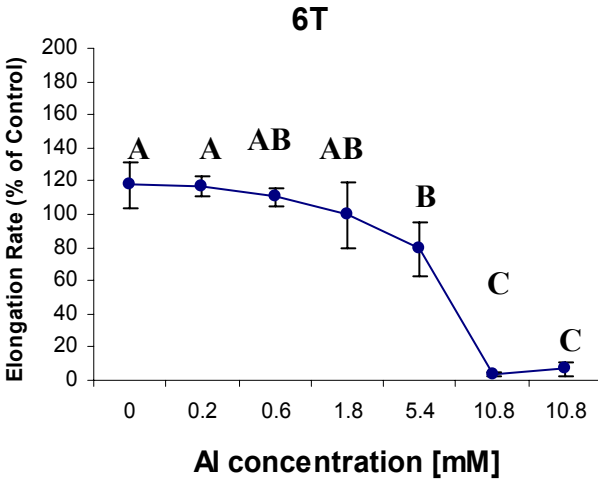
Figure 3. The stem elongation rate of *Spartina alterniflora* genotypes growing in a control hydroponic system and in a system of increasing Al concentration. Every two weeks the concentration was increased. Six genotypes are shown: five wild ecotypes that survived the brown-marsh die-off (11T, 6T, 3D, 7D, and 16D) and a commercial variety (Vermillion). The error bars are ±1 standard error. The different letters indicate significantly different means ($p<0.05$).


11T
Elongation Rate (% of Control)
200
180
160
140
120
100
80
60
40
20
0
A A B AB AB C C
0 0.2 0.6 1.8 5.4 10.8 10.
Al concentration [mM]


3D
Elongation Rate (% of Control)
200
180
160
140
120
100
80
60
40
20
0
AB A AB AB AC CD CD
0 0.2 0.6 1.8 5.4 10.8 10.8
Al concentration [mM]


VERMILLION
Elongation Rate (% of Control)
200
180
160
140
120
100
80
60
40
20
0
A A B A AC AC C
0 0.2 0.6 1.8 5.4 10.8 10.8
Al concentration [mM]


7D
Elongation Rate (% of Control)
200
180
160
140
120
100
80
60
40
20
0
A A A A B BC C
0 0.2 0.6 1.8 5.4 10.8 10.8
Al concentration [mM]


6T
Elongation Rate (% of Control)
200
180
160
140
120
100
80
60
40
20
0
A A AB AB B C C
0 0.2 0.6 1.8 5.4 10.8 10.8
Al concentration [mM]

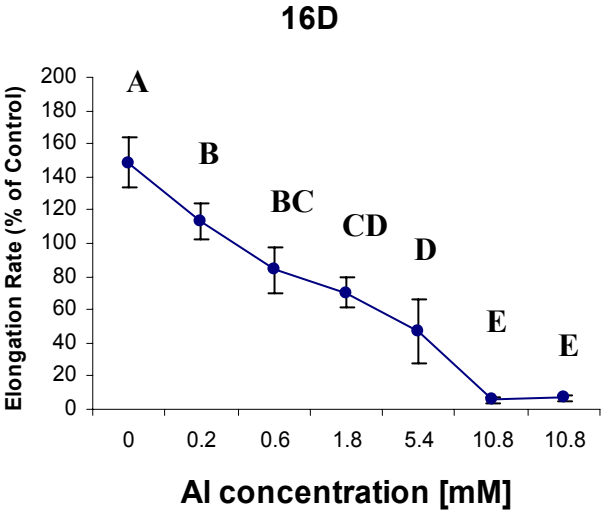

16D
Elongation Rate (% of Control)
200
180
160
140
120
100
80
60
40
20
0
A B BC CD D E E
0 0.2 0.6 1.8 5.4 10.8 10.8
Al concentration [mM]

Table 2. The Al concentration (mM) indicating the first significant decrease from the highest levels in stem elongation rate.

Genotype	First Significant Decrease (mM)
16D	0.2
11T	0.6
Vermillion	1.8
7D	5.4
6T, 3D	10.8 (interval 1)

2 Cumulative Stem Height

The cumulative stem height, averaged over genotype, significantly decreased with increased [Al] (Figure 2B, Table 3). The significant genotype x concentration interaction indicated that the effect of increasing [Al] on cumulative stem height differed with genotype (Table 3). Genotypes Vermillion, 7D, 16D and 3D showed a decrease in the cumulative stem height with increasing [Al] over the time interval (Figure 4). Genotype 11T and 6T increased stem elongation at 0.2 mM then decreased at higher [Al]s (Figure 4). Genotypes 16D and 6T were the most sensitive; 11T was intermediate; and 3D, 7D, and Vermillion were the least sensitive (Table 4).

Table 3. The results of an analysis of variance type 3 test of the fixed effects of aluminum concentration based on cumulative stem height.

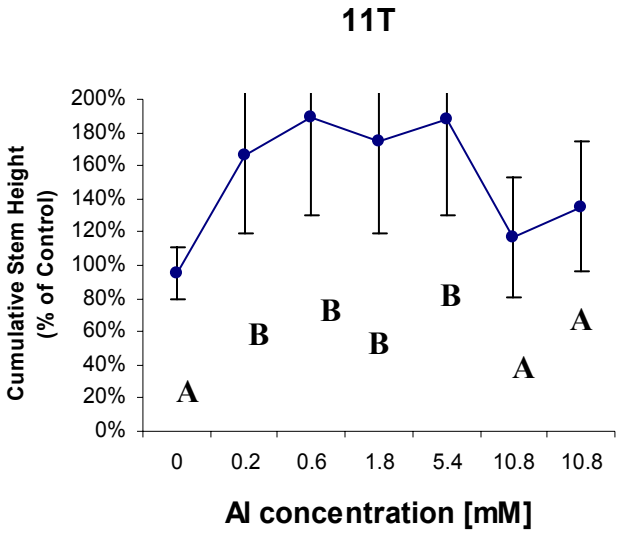
Effect	Numerator DF	Denominator DF	F Value	Prob > F
Genotype	5	5.99	3.12	0.0998
Concentration	6	42.2	11.79	<0.0001**
Genotype x Concentration	30	34	2.71	0.0028**

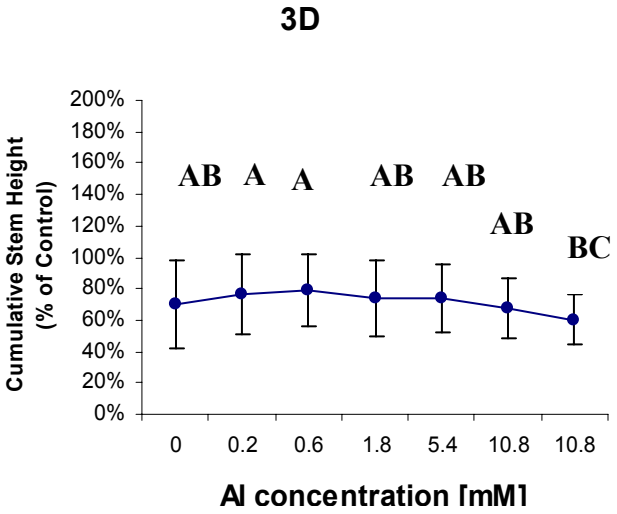
** Significantly different $p<0.01$

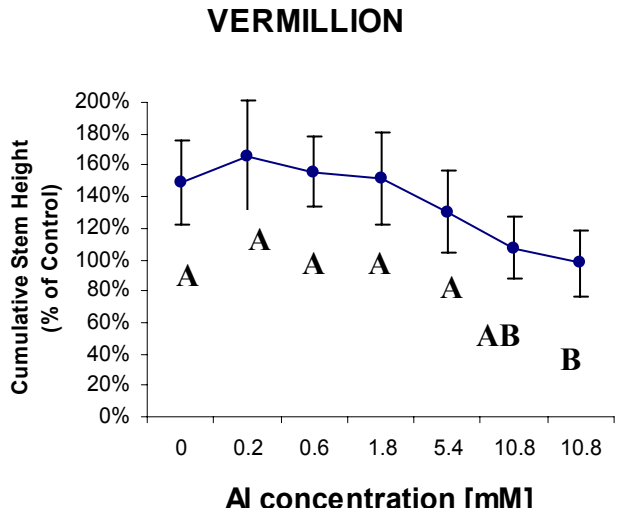
Table 4. The Al concentration (mM) indicating the first significant decrease from the highest levels in cumulative stem height.

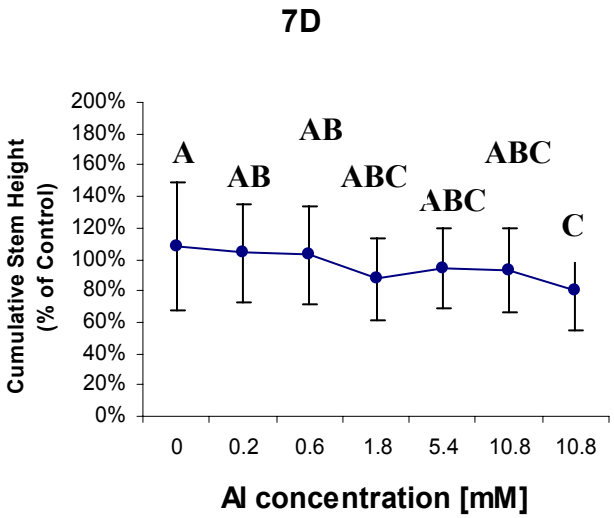
Genotype	First Significant Decrease (mM)
16D, 6T	5.4
11T	10.8 (interval 1)
7D, Vermillion, 3D	10.8 (interval 2)

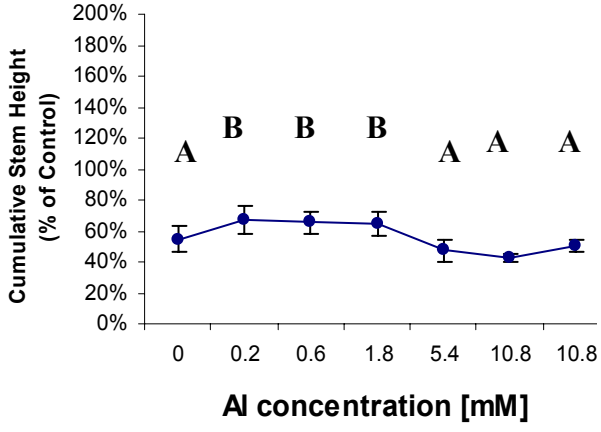
Figure 4. The cumulative stem height of *Spartina alterniflora* genotypes growing in a control hydroponic system and in a system of increasing Al concentration. Every two weeks the concentration was increased. Six genotypes are shown: five wild ecotypes that survived the brown-marsh die-off (11T, 6T, 3D, 7D, and 16D) and commercial variety (Vermillion). The error bars are ±1 standard error. The different letters indicate significantly different means ($p<0.05$).

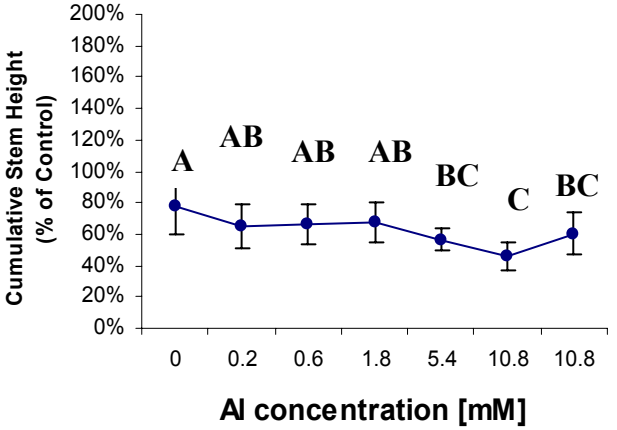

11T
Cumulative Stem Height (% of Control)
200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
A
B
B
B
B
A
A
0
0.2
0.6
1.8
5.4
10.8
10.8
Al concentration [mM]


3D
Cumulative Stem Height (% of Control)
200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
AB
A
A
AB
AB
AB
BC
0
0.2
0.6
1.8
5.4
10.8
10.8
Al concentration [mM]


VERMILLION
Cumulative Stem Height (% of Control)
200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
A
A
A
A
A
AB
B
0
0.2
0.6
1.8
5.4
10.8
10.8
Al concentration [mM]


7D
Cumulative Stem Height (% of Control)
200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
A
AB
AB
ABC
ABC
ABC
C
0
0.2
0.6
1.8
5.4
10.8
10.8
Al concentration [mM]


6T
Cumulative Stem Height (% of Control)
200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
A
B
B
B
A
A
A
0
0.2
0.6
1.8
5.4
10.8
10.8
Al concentration [mM]


16D
Cumulative Stem Height (% of Control)
200%
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
A
AB
AB
AB
BC
C
BC
0
0.2
0.6
1.8
5.4
10.8
10.8
Al concentration [mM]

a. Relative Growth Rate Based on Cumulative Stem Height. The relative growth rate (cm cm^{-1} day^{-1}) based on cumulative stem height, differed significantly among genotypes and with treatment (with or without Al) (Table 5). The Al treatment resulted in a significantly lower relative growth rate based on cumulative stem height compared to the control when averaged over all genotypes and concentrations (Al=0.101±0.016, control= 0.113±0.026). When averaged over treatment and concentrations, the relative growth rate based on cumulative stem height was significantly highest in genotype 16D and lowest in genotype 7D (Table 6). The genotype x treatment interaction was significant, indicating that the effect of the Al treatment varied with genotype. The relative growth rate based on cumulative shoot height of the genotype Vermillion was significantly lower with Al exposure, while the other genotypes showed no significant difference between treatment and control plants (Table 7). In addition, the main effect of aluminum concentration was significant (Table 5) with relative growth rate based on cumulative stem height significantly greater at the beginning of the experiment when [Al] was zero than at later experimental intervals when the [Al] concentrations where greater (Table 8). Figure 5 presents the relative growth rate based on cumulative stem height for all genotypes at all concentrations for both treatment and control.

Table 5. The results of an analysis of variance type 3 test of the fixed effects of aluminum concentration on relative growth rate based on cumulative stem height.

Effect	Numerator DF	Denominator DF	F Value	Prob > F
Genotype	5	29.6	3.97	0.0071**
Treatment	1	29.6	6.62	0.0153*
Genotype x Treatment	5	29.6	2.59	0.0467*
Concentration	5	136	17.98	<0.0001**
Genotype x Concentration	25	136	1.34	0.1457
Treatment x Concentration	5	136	2.19	0.0591
Genotype x Treat. x Conc.	25	136	1.21	0.2404

*Significantly different $p<0.05$
** Significantly different $p<0.01$

Table 6. The effect of genotype, averaged over concentration and treatment, on relative growth rate based on cumulative stem height (cm $cm^{-1}day^{-1}$) (n=48). The different letters indicate significantly different means (p>0.05). SE = ± 1 standard error.

	(cm $cm^{-1}day^{-1}$)		
Genotype	Mean	SE	Difference
11T	0.0165	±0.0080	CD
V	0.0197	±0.0028	AB
6T	0.0197	±0.0028	ABC
3D	0.0166	±0.0022	BCD
7D	0.0147	±0.0020	D
16D	0.0202	±0.0023	A

Table 7. The mean relative growth rate based on cumulative stem height (cm $cm^{-1}day^{-1}$) by treatment for six genotypes of *Spartina alterniflora* (n=24). The different letters indicate significantly different means among genotypes and between control and Al treatments (p<0.05). SE = ± 1 standard error.

	(cm cm^{-1} day^{-1})	Al		(cm cm^{-1} day^{-1})	Control	
Genotype	Mean	SE	Difference	Mean	SE	Difference
11T	0.0183	±0.0048	BCDE	0.0147	±0.0113	DEF
V*	0.0158	±0.0049	DEF	0.0235	±0.0039	A
6T	0.0191	±0.0050	ABCD	0.0203	±0.0039	ABCDE
3D	0.0160	±0.0038	EF	0.0173	±0.0031	ABCDE
7D	0.0134	±0.0025	F	0.0161	±0.0029	CDEF
16D	0.0187	±0.0039	ABC	0.0218	±0.0033	AB

*Significantly different <0.05

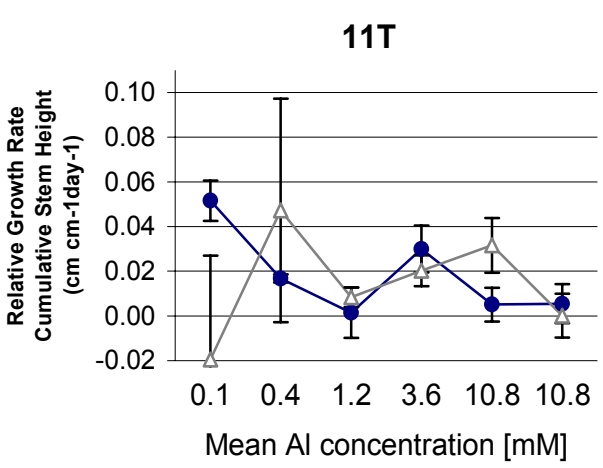
Table 8. The relative growth rate based on cumulative stem height (cm $cm^{-1}day^{-1}$) for each mean Al concentration averaged over treatment and genotype (n=48). The different letters indicate significantly different means (p<0.05). The mean Al exposure concentrations for each two week interval are presented. SE = ± 1 standard error.

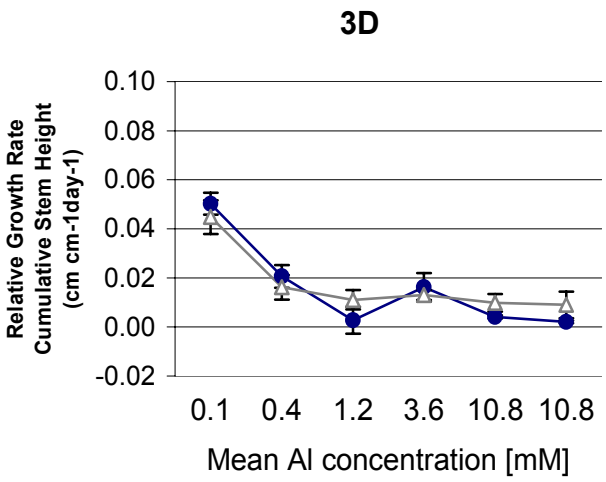
Relative growth rate based on cumulative stem height						
Mean Al (mM)	0.1	0.4	1.2	3.6	10.8	10.8
Mean (cm $cm^{-1}day^{-1}$)	0.0426	0.0218	0.0121	0.0140	0.0115	0.0054
Difference	A	B	CD	BC	CD	D
SE	±0.0048	±0.0040	±0.0019	±0.0020	±0.0020	±0.0029

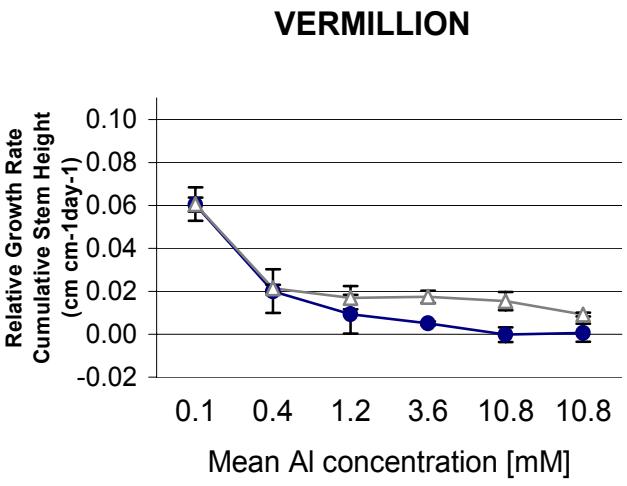
3 Stem Count

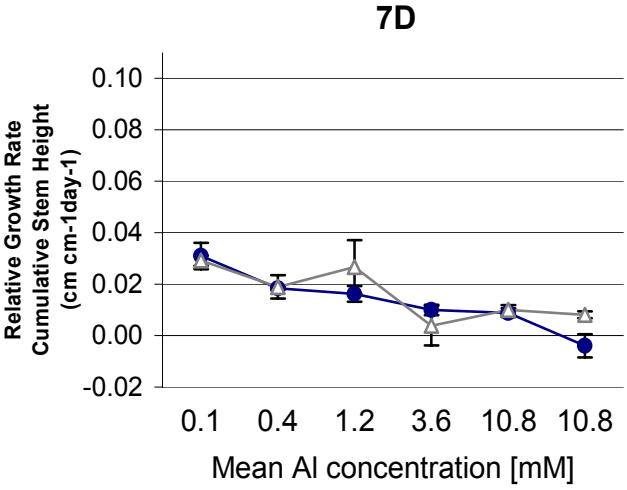
The stem count measured as a percent of the control, did not vary greatly with increasing [Al], although there was a significant concentration effect (Figure 2C, Table 9). One genotype

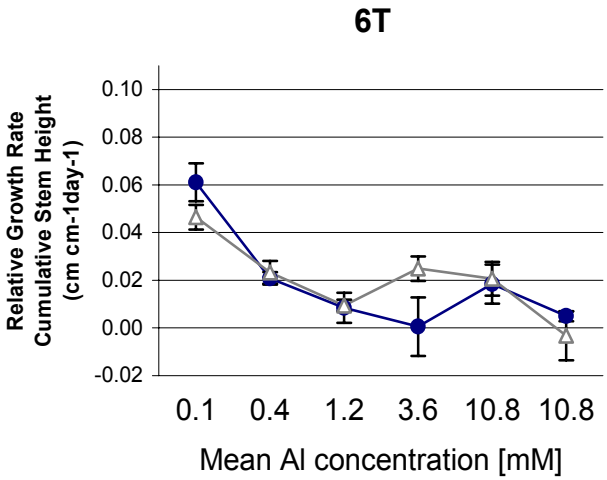
Figure 5. The relative growth rate (cm $cm^{-1}day^{-1}$) based on cumulative stem height of *Spartina alterniflora* genotypes growing in a control hydroponic system and in a system of increasing Al concentration. Al treatments received increasing Al concentrations every two weeks. The solid circles are from experimental (●) Al treatments and the hollow triangles denote the control group (△). Every two weeks the Al concentration was increased as follows: 0, 0.2, 0.6, 1.8, 5.4, 10.8, 10.8 mM. The mean Al exposure concentrations for each two week interval are plotted on the x-axis. Six genotypes are shown: five wild ecotypes that survived the brown-marsh die-off (11T, 6T, 3D, 7D, and 16D) and a commercial variety (Vermillion). The error bars are ±1 standard error.

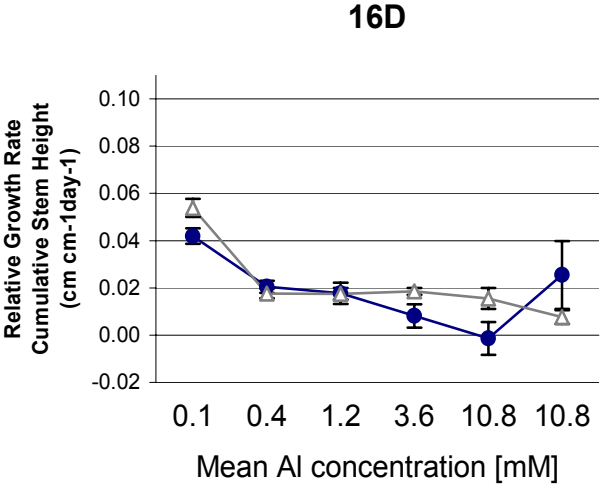

11T
Relative Growth Rate
Cumulative Stem Height
(cm cm-1day-1)
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
0.1 0.4 1.2 3.6 10.8 10.8
Mean Al concentration [mM]


3D
Relative Growth Rate
Cumulative Stem Height
(cm cm-1day-1)
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
0.1 0.4 1.2 3.6 10.8 10.8
Mean Al concentration [mM]


VERMILLION
Relative Growth Rate
Cumulative Stem Height
(cm cm-1day-1)
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
0.1 0.4 1.2 3.6 10.8 10.8
Mean Al concentration [mM]


7D
Relative Growth Rate
Cumulative Stem Height
(cm cm-1day-1)
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
0.1 0.4 1.2 3.6 10.8 10.8
Mean Al concentration [mM]


6T
Relative Growth Rate
Cumulative Stem Height
(cm cm-1day-1)
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
0.1 0.4 1.2 3.6 10.8 10.8
Mean Al concentration [mM]


16D
Relative Growth Rate
Cumulative Stem Height
(cm cm-1day-1)
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
0.1 0.4 1.2 3.6 10.8 10.8
Mean Al concentration [mM]

exhibited increased stem counts with increasing [Al] (6T), while another genotype had decreased stem counts (Vermillion), and one genotype did not significantly change with increasing [Al] (3D) (Figure 6). Two genotypes (11T and 16D) showed a decrease in stem count at an elevated [Al] (10.8 internal 1), but then recovered at the second interval of 10.8 mM Al. Because of this recovery, stem counts for 11T and 16D are best considered as not changing with [Al]. Table 10 presents the Al sensitivities based on the first significant decrease approach. Hence, the genotypes widely varied in stem count response, some decreased while others increased (Table 9 - significant genotype by concentration effect).

Table 9. The results of an analysis of variance type 3 test of the fixed effects for stem count.

Effect	Numerator DF	Denominator DF	F Value	Prob > F
Genotype	5	6.93	2.51	0.1320
Concentration	6	57.7	2.83	0.0175*
Genotype x Concentration	30	42.6	2.60	0.0021**

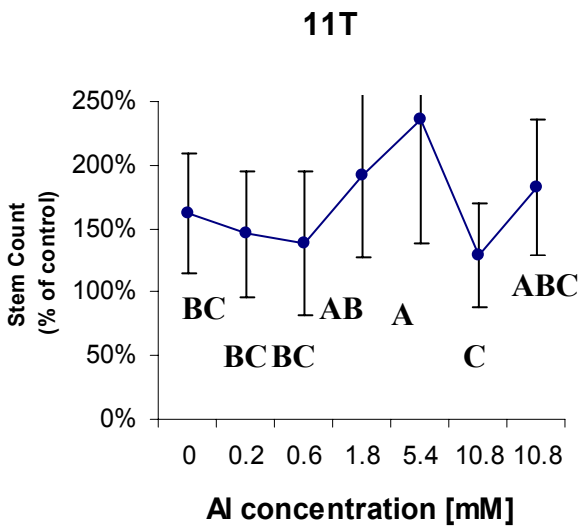
*Significantly different $p<0.05$
** Significantly different $p<0.01$

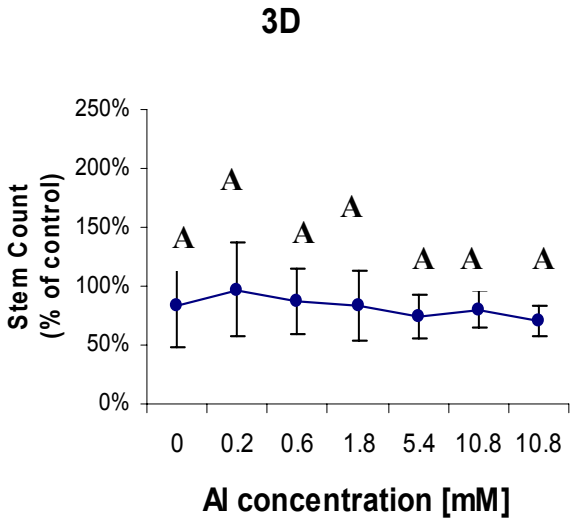
Table 10. The aluminum concentration (mM) where the first significant decrease from the highest levels in stem count was observed.

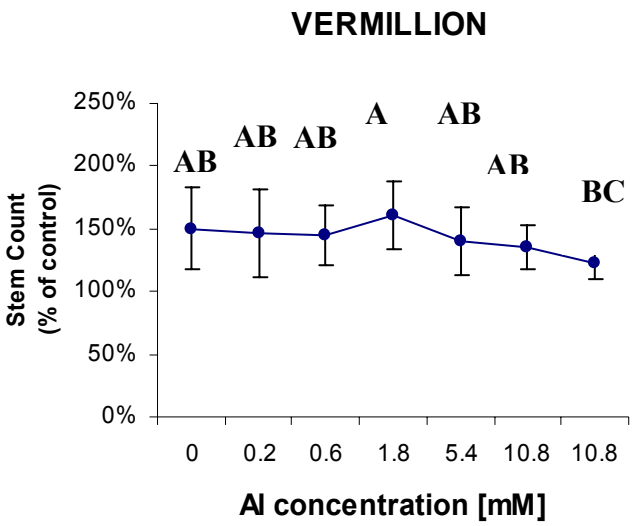
Genotype	First significant decrease concentration (mM)
Vermillion, 7D,	10.8 (interval 2)
3D, 11T, 16D	no change
6T	Significant increase

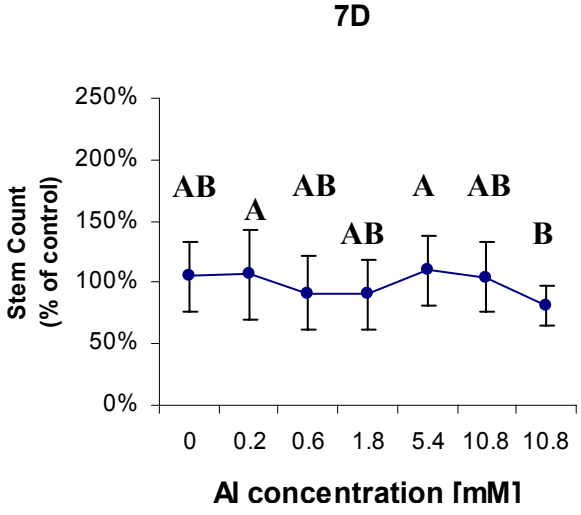
a. Relative Growth Rate Based on Stem Count. The relative growth rate based on stem count (stem $stem^{-1}day^{-1}$) (Figure 7) showed only a significant concentration effect (Table 11). The relative growth rate based on stem count was significantly greater during the period from four to six weeks, when the Al treatment was held at zero, compared to the treatment periods in which [Al]s were increased (Table 12). High variation in this parameter prevented it from being a valuable indicator of growth response to Al stress.

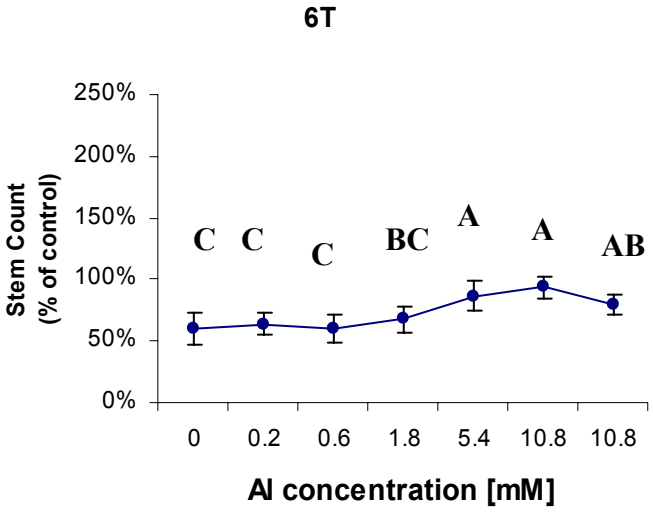
Figure 6. The number of *Spartina alterniflora* plant stems counted in each in the control hydroponic system and in a system of increasing Al concentration. Every two weeks the concentration was increased until 10.8 mM. Six genotypes are shown: five wild ecotypes that survived the brown-marsh die-off (11T, 6T, 3D, 7D, and 16D) and a commercial variety (Vermillion). The error bars are ±1 standard error. The different letters indicate significantly different means ($p<0.05$).


11T
Stem Count (% of control)
250%
200%
150%
100%
50%
0%
BC
BC BC
AB
A
C
ABC
0 0.2 0.6 1.8 5.4 10.8 10.8
Al concentration [mM]


3D
Stem Count (% of control)
250%
200%
150%
100%
50%
0%
A
A
A
A
A
A
0 0.2 0.6 1.8 5.4 10.8 10.8
Al concentration [mM]


VERMILLION
Stem Count (% of control)
250%
200%
150%
100%
50%
0%
AB
AB AB
A
AB
AB
BC
0 0.2 0.6 1.8 5.4 10.8 10.8
Al concentration [mM]


7D
Stem Count (% of control)
250%
200%
150%
100%
50%
0%
AB
A
AB
AB
A
AB
B
0 0.2 0.6 1.8 5.4 10.8 10.8
Al concentration [mM]


6T
Stem Count (% of control)
250%
200%
150%
100%
50%
0%
C C
C
BC
A
A
AB
0 0.2 0.6 1.8 5.4 10.8 10.8
Al concentration [mM]

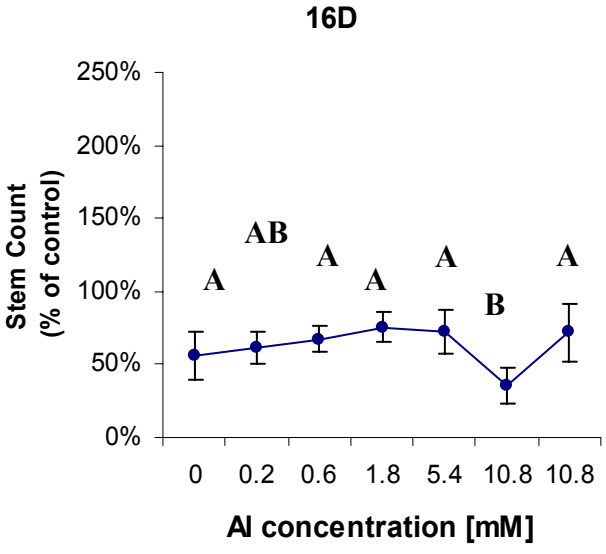
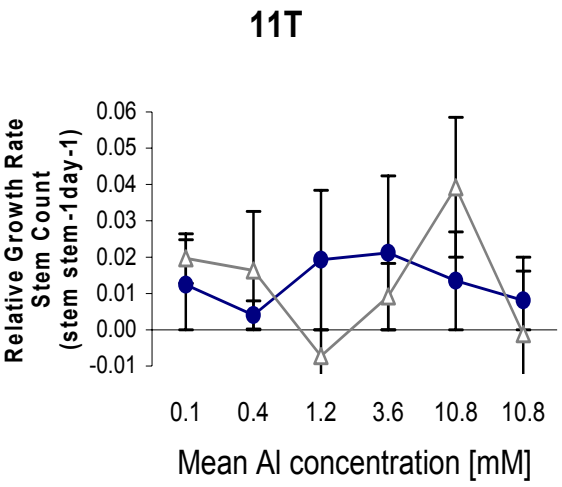
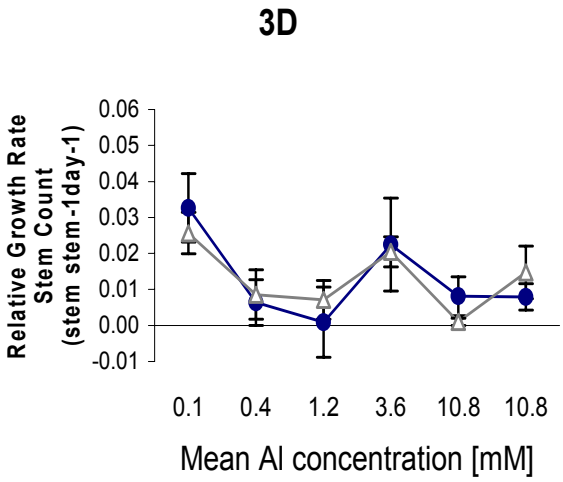
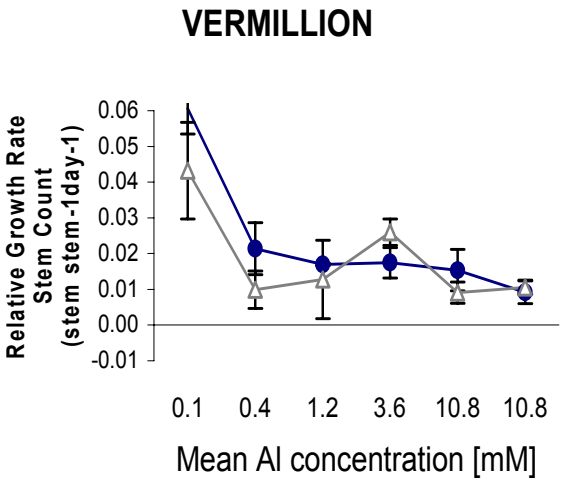
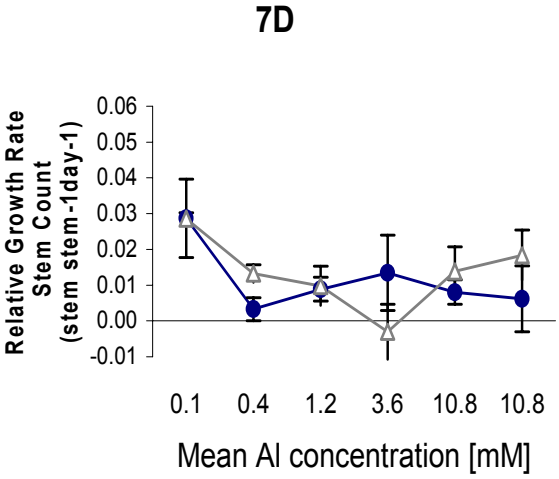

16D
Stem Count (% of control)
250%
200%
150%
100%
50%
0%
A
AB
A
A
A
B
A
0 0.2 0.6 1.8 5.4 10.8 10.8
Al concentration [mM]

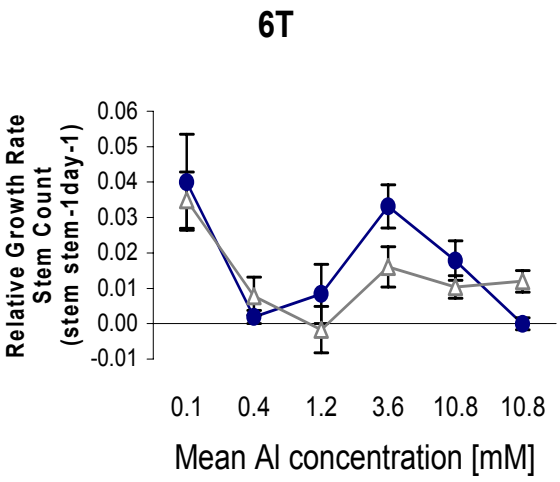
Figure 7. The relative growth rate based on the stem count (stem $stem^{-1}day^{-1}$) of *Spartina alterniflora* genotypes growing in a control hydroponic system and in a system of increasing Al concentration. The solid circles are experimental (●), the hollow triangles denote the control group (△). Every two weeks the concentration was increased until 10.8 mM Al as follows: 0, 0.2, 0.6, 1.8, 5.4, 10.8, 10.8 mM. The mean Al exposure concentrations for each two week interval are plotted on the x-axis. Six genotypes are shown: five wild ecotypes that survived the brown-marsh die-off (11T, 6T, 3D, 7D, and 16D) and a commercial variety (Vermillion). The error bars are ±1 standard error.


11T
Relative Growth Rate
Stem Count
(stem stem-1day-1)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
0.1
0.4
1.2
3.6
10.8
10.8
Mean Al concentration [mM]


3D
Relative Growth Rate
Stem Count
(stem stem-1day-1)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
0.1
0.4
1.2
3.6
10.8
10.8
Mean Al concentration [mM]


VERMILLION
Relative Growth Rate
Stem Count
(stem stem-1day-1)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
0.1
0.4
1.2
3.6
10.8
10.8
Mean Al concentration [mM]


7D
Relative Growth Rate
Stem Count
(stem stem-1day-1)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
0.1
0.4
1.2
3.6
10.8
10.8
Mean Al concentration [mM]


6T
Relative Growth Rate
Stem Count
(stem stem-1day-1)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
0.1
0.4
1.2
3.6
10.8
10.8
Mean Al concentration [mM]

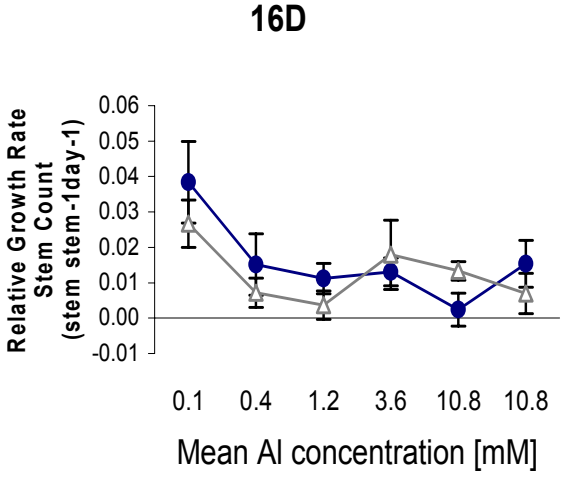

16D
Relative Growth Rate
Stem Count
(stem stem-1day-1)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
0.1
0.4
1.2
3.6
10.8
10.8
Mean Al concentration [mM]

Table 11. The results of analysis of variance type 3 tests of fixed effects for relative growth rate of stem count

Effect	Numerator DF	Denominator DF	F Value	Prob > F
Genotype	5	35.7	0.88	0.5033
Treatment	1	35.7	0.00	1.0000
Genotype x Treatment	5	35.7	1.61	0.1821
Concentration	5	176	12.69	<0.0001**
Genotype x Concentration	25	176	1.13	0.3125
Treatment x Concentration	5	176	0.87	0.5046
Genotype x Treat. x Conc.	25	176	0.93	0.5610

** Significantly different $p<0.01$

Table 12. The relative growth rate based on stem count for each aluminum concentration averaged over the six genotypes of *Spartina alterniflora* (control and with Aluminum) (n=48) (stem stem^{-1}day^{-1}). The different letters indicate significantly different means ($p<0.05$). The mean Al exposure concentrations for each two week interval are presented.

Relative growth rate based on stem count						
Mean mM Al	0.1	0.4	1.2	3.6	10.8	10.8
(stem stem^{-1}day^{-1}) Mean	0.0294	0.0086	0.0069	0.0169	0.0117	0.0087
Difference	A	C	C	B	BC	C
SE	±0.0091	±0.0066	±0.0072	±0.0081	±0.0080	±0.0076

4 Biomass

The root and stem biomass, as well as the total biomass (root plus stem), when averaged over genotype, were significantly lower in the Al treatments relative to the controls (Tables 13 and 14). However, an analysis of the significant genotype by treatment interaction for total biomass and for stem biomass (Table 14) indicated that not all genotypes responded similarly to the Al dosage (Table 14). The Vermillion, 6T, 3D, 7D, and 16D genotypes had significantly lower stem and total biomass when exposed to Al compared to no Al exposure. In contrast, the biomass production of genotype 11T was not significantly affected by [Al] (Tables 13 and 14). The effect of Al on root mass did not statistically differ with genotype, but did differ with treatment (Tables 13 and 14).

Table 13. The net (A) root, (B) stem, and (C) total biomass (g) of *Spartina alterniflora* genotypes growing in a control hydroponic system and in a system of increasing Al concentration. The values are means and ±1 standard error of 4 replications. Five wild genotypes (11T, 6T, 3D, 7D, and 16D), which had survived the brown-marsh die-off, and commercial variety (Vermillion) were dosed with Al (n=4). The different letters indicate significant differences between Al and control treatments, averaged over genotypes ($p<0.05$). (Root mass has no significant treatment x genotype effect; however, no comparison of these means were made.) † The Al treatment was significantly different from the control, which was equal to 100% ± 6.4-7.6 %.

(A) Root Biomass					% control	
Genotype (n=4)	Al (g)	SE	control	SE		SE
11T	91.7	±27.6	97.6	±19.5	94%	±28
Vermillion	98.5	±17.7	283.7	±64.7	35%	±6
6T	82.4	±24.5	274.9	±68.5	30%	±8
3D	47.5	±12.0	209.2	±59.9	23%	±5
7D	72.4	±28.5	288.2	±46.2	25%	±9
16D	77.0	±20.0	273.7	±17.2	28%	±7
Treatment mean (n=24)	78.2 A	±21.7	237.9B	±46.0	39.1%	± 11.1 †
(B) Stem Biomass					% control	
Genotype (n=4)	Al (g)	SE	control	SE		SE
11T	38.0 CD	±8.2	59.9 BCD	±10.4	63%	±13
Vermillion*	46.6 CD	±8.8	173.6 A	±36.4	27%	±5
6T	43.6 CD	±11.2	132.5 ABC	±24.3	33%	±8
3D*	30.8 D	±12.8	134.6 AB	±29.2	23%	±9
7D*	39.5 CD	±11.8	146.0 AB	±27.7	27%	±8
16D*	42.6 CD	±14.1	204.5 A	±18.4	21%	±9
Treatment mean (n=24)	40.2 A	± 11.1	141.9 B ±24.4		32.3%	± 51.6 †
(C) Total Biomass					% control	
Genotype (n=4)	Al (g)	SE	control	SE		SE
11T	129.7 CD	±34.3	157.4 BCD	±25.6	82%	±21
Vermillion*	145.0 CD	±25.7	457.3 A	±101.0	32%	±5
6T*	125.9 CD	±23.1	407.5 AB	±74.3	31%	±5
3D*	78.3 D	±23.0	343.7 ABC	±81.8	23%	±6
7D*	111.9 CD	±40.3	434.2 AB	±67.7	26%	±9
16D*	119.6 CD	±32.7	478.2 A	±18.4	25%	±6
Treatment mean (n=24)	118.4 A	± 29.9	379.7 B	±61.5	36%	± 9.3 †

*Significantly different $p<0.05$

Table 14. ANOVA table for *Spartina alterniflora* biomass (g) expressed as mass (A) root, (B) stem, and (C) total.

(A) Root Biomass					
Source	Numerator DF	Denominator DF	Sum of squares	F ratio	Prob>F
Treatment	1	1	305842	50.1022	<0.0001**
Genotype	5	5	57742	1.8918	0.1201
Treatment x Genotype	5	5	59851	1.9609	0.1083
(B) Stem Biomass					
Source					
Treatment	1	1	124003	78.1616	<0.0001**
Genotype	5	5	26198	3.3026	0.0148*
Treatment x Genotype	5	5	21671	2.7319	0.0342*
(C) Total Biomass					
Source					
Treatment	1	1	819332	73.1402	<0.0001**
Genotype	5	5	149344	2.6663	0.0377*
Treatment x Genotype	5	5	141561	2.5274	0.0464*

*Significantly different $p<0.05$
** Significantly different $p<0.01$

When biomass response to Al was expressed as a percent of the control, only the overall Al treatment effect was significant (Table 15). The main effect of genotype and the interaction of genotype with treatment were not significant (Table 15). Thus, Al did reduce the root, stem, and total biomass of the *Spartina alterniflora* relative to the controls regardless of genotype. Although the effect of Al did not statistically differ with genotype, genotype 11T consistently had less of a reduction in biomass relative to the controls when exposed to Al. This result tends to support the stem and total biomass findings of a lesser Al effect in this genotype compared to the other genotypes.

5 Comparison of Indices of Plant Growth Response to Aluminum

The response of the genotypes to increasing Al varied depending on growth parameter measured (Table 16). Growth parameters which had no significant genotype effect were biomass and relative growth rate based on stem count and were thus not presented in Table 16. Genotype

Vermillion was intermediate compared to the other five genotypes (16D, 11T, 7D, 6T, and 3D).

Table 15. ANOVA table for *Spartina alterniflora* biomass (g) expressed as a percent of the control treatment (A) root, (B) stem, and (C) total.

(A) Root Biomass (% control)					
Source	Numerator DF	Denominator DF	Sum of squares	F ratio	Prob>F
Treatment	1	1	44501	35.52	<0.0001**
Genotype	5	5	7403	1.18	0.3371
Treatment x Genotype	5	5	7403	1.18	0.3371
(B) Stem Biomass (% control)					
Source					
Treatment	1	1	54946	66.48	<0.0001**
Genotype	5	5	2501	0.60	0.6961
Treatment x Genotype	5	5	2501	0.60	0.6961
(C) Total Biomass (% control)					
Source					
Treatment	1	1	48493	55.38	<0.0001**
Genotype	5	5	5193	1.18	0.3352
Treatment x Genotype	5	5	5193	1.18	0.3352

** Significantly different $p<0.01$

Table 16. Genotypes ranked in order of increasing tolerance. Genotype 7D had the lowest relative growth rate based on cumulative stem height (RGR_{CSH}), yet the highest cumulative stem height. Growth parameters which had no significant genotype effect were relative growth rate based on stem count (RGR_{SC}) and biomass.

Growth parameter	Increasing Al tolerance
Stem elongation	16D > 11T > Vermillion > 7D > 6T, 3D
Cumulative stem height	16D, 6T > 11T > 7D, Vermillion, 3D
RGR_{CSH}	6T > 16D > 11T > 3D > Vermillion > 7D
Stem count	11T, 6T, 16D, 3D > Vermillion, 7D
RGR_{SC}	no effect
Biomass	no effect

CHAPTER 5

DISCUSSION

After reviewing the literature (see Chapter 2, Background), I conclude that it is reasonable to assume that a combination of drought and pyrite oxidation may provide the soil physio-chemical conditions necessary to cause [Al] to increase. Thus I postulated that plant toxicity resulting from high [Al] may be relevant to understanding the mortality of wetland vegetation, and in particular, on one occasion in Louisiana, the brown-marsh phenomenon. The results of this thesis may be useful should a similar condition occur in the future.

The goal of this research was to provide incite into the over-arching hypothesis: that an increase of [Al], or availability, causes toxicity to *Spartina alterniflora,* the primary species of coastal fringe wetlands along the Atlantic and Gulf coasts of the United States. If mortality of *Spartina alterniflora* does occur due to high [Al], then it would suggest that high [Al] could be a cause or contributing factor for the brown-marsh event given the elevated Al that occurred in brown marsh sites (Mckee *et al.* 2004). Currently, there is no evidence for mortality of *Spartina alterniflora* due to high [Al]. If mortality does not occur outright, a further goal is to determine at what level [Al] exhibits a toxic effect.

Secondly, an attempt was made to screen genotypes of *Spartina alterniflora* for variability in Al tolerance. Patches of different genotypes that remained alive at the brown-marsh sites and a commercial variety, Vermillion, were screened. A unique methodology was employed to determine if individual cultivars among a single species could be screened for variability based upon the measurements proposed in this study. If whole plant growth parameters are useful for screening different cultivars of a single species for [Al] tolerance, then growth parameters may be used to predict resistance variability in genotypes of *Spartina alterniflora*.

Stem elongation rate, cumulative stem height, stem count, and biomass were compared for determining variability among genotypes of *Spartina alterniflora* in Al tolerance. A comparison of the measured growth responses could determine whether the measured parameters denote resistance variability in genotypes.

1 Stem Elongation Rate

Stem elongation has been used as a reliable indicator of *Spartina alterniflora* response to sub-lethal Cd toxicity (Mendessohn *et al.* 2001). Stem elongation rate was useful in determining the effect of [Al] on *Spartina alterniflora*, as evidenced by the fact that the effect of increasing [Al] on stem elongation differed with genotype (Table 1). Significant differences in the growth rate of the genotypes were found when [Al] increased. Models were used to interpret dose-response curves in order to differentiate between genotypes. Dose response curves were also used to establish theoretical critical toxicity thresholds for the genotypes. The first significant decrease in growth rate as [Al] increased was determined to be an accurate method for establishing concentration thresholds for the genotypes. Thorton's critical toxicity level or threshold could only be assessed for the stem elongation data because Thorton's critical level requires, at a minimum, a 70 percent reduction in the growth rate of dosed plants compared to controls (Lux and Cumming 1999). The first significant decrease in the growth rate had the best resolution for accurately differentiating genotypes.

a. Dose-Response Curves. Aluminum dose-response curves, generated from stem elongation rate data, are exemplified by models of Al dose-response curves found in the botanical literature. Barceló and Poschenreider (2002) characterized at least three models of root growth rate responses related to the toxic effect of [Al] as different models of toxicity (Figure 8). The models of toxicity were described as: (A) threshold for toxicity: the effect is minimal until a toxicity

threshold is reached, (B) hormesis model: a stimulation effect at a lower dose or a shorter exposure time, and (C) threshold for tolerance: an increase in growth rate occurs after an initial period of reduced growth.

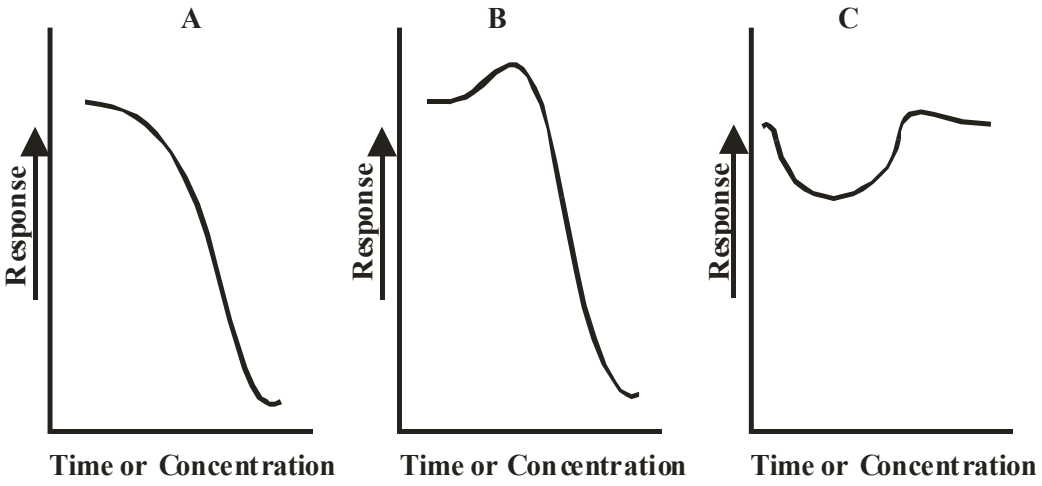


Figure 8. Three possible dose-response curves for plants are shown. The three curves show: (A) threshold for toxicity or when toxicity occurs through the concentration range or time interval, (B) hormesis or stimulation at low doses or short time interval, (C) threshold for tolerance or tolerance is expressed after a lag phase (adapted from Barceló and Poschenreider (2002)).

The toxicity threshold model implies that there is no recovery from damage and an increasing toxicity throughout the duration or concentration range. Five of the six genotypes (3D, 6T, 7D, 11T, and 16D) showed a response to increasing [Al] similar to the threshold for toxicity model (Figure 3). The threshold for tolerance model was not observed in any of the six genotypes studied. The Vermillion genotype showed a hormesis effect.

Aluminum may stimulate plant growth at low doses (Barceló and Poschenreider 2002). The response of the Vermillion genotype to [Al] could be considered an example of Al hormesis or stimulation at low doses or short exposure durations. Due to increasing [Al], the growth rate of Vermillion increased approximately 80 percent between 0.2 and 1.8 mM Al. At concentrations above 1.8 mM Al, the stem elongation rate rapidly decreased to zero (Figure 3). The initial

stimulation was followed by toxicity; thus, the stem elongation response for the Vermillion cultivar appears similar to Barceló and Poschenreider's (2002) hormesis model. Although stem elongation at 1.8 mM Al for Vermillion was significantly different ($p<0.05$) from the other concentrations, the standard error was greatest for this mean. Thus, the hormesis effect of Vermillion is questionable.

The stem elongation rate of all the experimental cultivars (n=24) showed a reduced growth rate with increasing [Al], which resembles the threshold for toxicity model (Figure 2a). The initial resistance changed to reduced growth rate above 1.8 mM Al. The stem elongation data in the context of the dose response curve models suggested two patterns of Al effect. Five of six genotypes exhibited a continuous decline in elongation rate; the Vermillion genotype may have been stimulated at low Al doses.

b. Critical Toxicity Level. Thorton's critical toxicity level is the concentration of toxic metal ion causing the growth indicators of the experimental treatments to drop to below 30 percent of the controls (Lux and Cumming 1999). The percent of control of the stem elongation rate decreased below 30 percent in all genotypes in my study, allowing the determination of critical toxicity levels for each genotype (Figure 2A and Figure 3). Genotypes 11T, 3D, Vermillion, 6T, and 16D had a critical toxicity level of 5.4 mM Al. Genotype 7D had a critical toxicity threshold that occurred after the second 10.8 mM Al dosing. Genotype 7D also had the maximum critical toxicity level. It is reasonable to estimate that the critical toxicity level for *Spartina alterniflora* is between 5.4 mM and 10.8 mM Al based on stem elongation rate expressed as a percent of the control (Figure 3). A narrower range in the concentrations for the dose interval may have increased the usefulness of the critical toxicity threshold, but the general target concentration range had to be determined before this could be done. I was able to make two separations in the

genotypes as described previously using the critical toxicity level model.

c. First Significant Decrease in Stem Elongation Rate. I was able to more accurately determine the relative tolerance of the genotypes to Al using the first significant decrease in stem elongation rate for each genotype. For stem elongation rate, the first significant decrease in the percent of control showed greater separation of the genotypes (Table 2) than either the dose response models or the critical toxicity level. The Vermillion genotype was the only genotype with a significant increase in stem elongation rate. However, the two different interpretations of data do not agree. For example, when the first significant decrease is used, genotype 7D would appear to be intermediate in tolerance, while the critical toxicity level indicates that it is the most tolerant. The results of the first significant decrease (Table 2) are not similar to the results of critical toxicity level or dose-response models. There were five groupings of the genotypes when the first significant decrease approach was used, rather than two groupings with the dose response curves and critical toxicity level.

2 Cumulative Stem Height

I used cumulative stem height in two ways to assess the effect of [Al] on genotype growth response: (A) cumulative stem height, i.e., total stem length per pot, was used as a surrogate for the biomass and expressed as a percent of the control, and (B) the change in cumulative stem height between successive sampling periods, i.e., [Al]s were used to calculate relative growth rates (cm cm^{-1} day^{-1}) (see Chapter 3, Materials and Methods). The inferences about the effects of [Al] on genotype tolerance varied with approach.

Most genotypes had significant decreases in cumulative stem height relative to control with increasing [Al], while some genotypes did not (Figure 4). The Al toxicity threshold concentration may be determined by comparing the [Al]s that resulted in the first significant

decrease in growth rate. There was a significant genotype x concentration effect of cumulative stem height (p=0.0028) (Table 3). It was evidenced that genotype variability can be determined by using the different concentration intervals where growth responses of genotypes had the first significant decrease in the percent of control based on the cumulative stem height (Table 4). The first significant decrease approach was used to rank genotypes in accordance with Al tolerance. More tolerant genotypes had higher concentrations.

a. Critical Toxicity Level and Dose-Response Models. Neither the dose-response models nor the critical toxicity levels were applicable to the cumulative stem height responses measured in this study. The requirement for a 70 percent decrease in growth response to apply the critical toxicity threshold model to the cumulative stem height data was not met. With respect to the dose-response models, the large variation in the data for some genotypes, e.g., 11T and 6T, made inferences difficult to make. For example, there appeared to be a stimulation or hormesis effect in cumulative stem height at low [Al]s for two of the genotypes (11T and 6T); although the standard errors for means overlap considerably. The other four genotypes (Vermillion, 3D, 7D, and 16D) exhibited decreased cumulative stem height with increasing [Al], which is a response similar to the threshold for toxicity model. The stem elongation rate of all the experimental cultivars combined (n=24) showed a slightly reduced stem height with increasing [Al] (Figure 2B), above 0.6 mM Al.

b. First Significant Decrease of Cumulative Stem Height. Because the genotype x concentration effect on cumulative stem height was significant (p=0.0028) (Table 3), I was able to use the genotype-differences in the first significant decrease in cumulative stem height to assess Al toxicity threshold concentrations (Table 4), as I did for the stem elongation data. Genotype 16D and 6T were the most sensitive genotypes to Al concentration. Genotypes

Vermillion, 3D, and 7D were the most tolerant or resistant (Table 4). Three distinct groupings may be made among the genotypes using the first significant decrease of cumulative stem height: sensitive, intermediate, and most resistant.

The genotype x concentration effect was statistically significant for both cumulative stem height and for stem elongation ($p=0.0028$ and $p=0.0177$, respectively). These significant interactions indicate that the genotypes are responding differently to increasing Al concentrations. The consistency of these results suggests that there were differences in genotype tolerance to Al. In contrast, the genotype x concentration effect and the genotype x treatment x concentration effect were not significant for relative growth rate of cumulative stem height.

c. Relative Growth Rate of Cumulative Stem Height. The relative growth rate based on cumulative stem height was significantly different among genotypes depending on the Al treatment (Al or control) ($p=0.0467$) (Table 5). Hence one genotype responded differently to the Al treatment from all of the others. This genotype was Vermillion, which had a significantly lower relative growth rate based on cumulative stem height when exposed to Al than when not (Table 7). The other genotypes showed no significant difference in relative growth rate based on cumulative stem height between control and Al-dosed plants. The treatment x concentration interaction was close to significant and thus indicates that over the duration of the experiment; the treatment plants, those exposed to Al, responded differently than the controls (Table 5 and 7). Overall, the relative growth rate based on cumulative stem height declined with increasing Al concentration (Table 8).

The significant genotype x treatment effect ($p=0.0467$) (Table 5) suggested that the variation in growth rate based on cumulative stem height was due to differences in tolerance to Al and not due to genetics (Table 5). However, some genotypes may have inherently greater

growth rates than others. The significant genotype effect (Table 5, p=0.0071) suggests that, regardless of whether the genotype received Al or not, some genotypes had a greater relative growth rate based on cumulative stem height than others. This result suggests that certain genotypes, e.g. 16D, 6T, and Vermillion (Table 6), innately have faster growth rates than the others. The significant genotype effect may indicate that some genotypes are predisposed to grow to various heights. Tall and short *Spartina alterniflora* varieties exist in the wild and could explain the significant genotype difference. Adams (1963) described three forms of *Spartina alterniflora*: tall, medium, and short. The reason for the variation in the height form has been debated, if height form is due to genetic or environmental reasons as outlined in Mooring *et al.* (1971) and Gallagher *et al.* (1988). Results of Mooring *et al.* (1971) demonstrated that salinity determined height and that genotype had no effect on height variation. The environment affected height variation, i.e., the height forms are ecophenes. Mendelssohn and Seneca (1980) found soil drainage to account for 70 percent of the variation in plant heights. Moreover, Gallagher *et al.* (1988) found that the height forms can maintain height differences for years when grown in a common environment.

Although there was no overall significant effect of genotype on cumulative stem height (Table 3), the overall genotype effect was significant for relative growth rate based on cumulative stem height (Table 5, Genotype (p=0.0071). The relative growth rate based on cumulative stem height data from my experiment results contradicted the findings of Mooring *et al.* (1971), who did not find genotypic differences in salt tolerance of *Spartina alterniflora.* The growth responses of the genotypes in the present research were likely due to both inherent genetic differences in the genotypes and to species-specific differences in response to Al. The

significant genotype x concentration and genotype by treatment interactions support this conclusion.

3 Stem Count

The stem count fluctuated upward with new tiller production and downward as mortality increased. New stems replaced the dead and, in this way, the stem count remained relatively constant (Figure 2C). The critical toxicity level model was not applicable because the stem count did not decrease below 70 percent of control. Also, dose-response models were not useful in describing the trends in stem count because the trends were only descending. Stem count decreased over time for some genotypes and increased for others (Figure 6, significant genotype x concentration effect (Table 9)). Because stem count had a significant genotype x concentration effect, the first significant decrease was utilized. Three separations in the genotypes based on Al tolerance were made with stem count data: increasing stem count, decreasing stem count, and no change.

The average stem count of all the experimental cultivars (n=24) shows a slight, but transitory, increase in growth with increasing [Al] or time interval (Figure 2C). At low [Al], the experimental plants grew at a rate equal to that of the control plants. Between 0.6 and 5.4 mM Al stem count increased approximately 25 percent. After this stimulation, the stem count decreased back to 100 percent of the control plants. Stem count was not significantly different at the genotype level. The effect of Al concentration on stem count was significant ($p=0.0175$) (Table 9). Similarly, the genotype x concentration effects on stem count were statistically significant for stem count ($p=0.0021$) (Table 9), as it was for cumulative stem height, and for stem elongation ($p=0.0028$ and $p=0.0177$, respectively). Thus, the effect of increasing [Al] on stem count did differ with genotype.

a. First Significant Decrease of Stem Count. The six genotypes may be separated into three distinct groupings: those which decreased, those that increased, and those with no change (Figure 6). The stem count of genotype 3D did not significantly increase or decrease and the graph does not resemble theoretical dose-response models. The stem counts of genotypes 11T and 16D, although exhibiting transitory increases and decreases, recovered and therefore these genotypes show little change in stem count given the large standard errors. For genotype 6T stem count increased and there was no first significant decrease. Genotypes Vermillion and 7D did not significantly decrease until the second interval at 10.8mM Al.

b. Relative Growth Rate of Stem Count. The treatment effects on relative growth rate (cm cm^{-1} day^{-1}) based on stem count was largely insignificant (Table 11). Differences in genotype x concentration based on stem count contrasted with the results of the genotype x concentration based on the relative growth rate of stem count. The relative growth rate based on stem count only had a significant Al effect (concentration), and it was not useful in differentiating genotypes. For relative growth rate based on stem count, the significant difference of the concentration effect was not as highly significant as it was for stem count (Table 11). Also, there were no significant differences in any of the statistical treatments with the exception of concentration.

4 Biomass

Root, stem, and total biomass, expressed as dry weight in grams and percentage of control, decreased due to Al; thus, there were significant treatment effects (Tables 13, 14 and 15). Genotype and genotype x treatment interactions were significant for stem and total biomass (Table 14). In contrast, biomass expressed as percent of control revealed no significant genotype or genotype x treatment effects (Table 15). For root mass there was neither a significant

genotype effect nor a significant genotype x treatment effect (Tables 14 and 15). Roots had grown out of their individual pots and intertwined, making it impossible to separate the roots by genotype. Escaping roots may explain why the root biomass was not significant.

Macedo *et al.* (1997) found weight parameters to be better than length measurements for distinguishing toxicity thresholds for screening genotypes of rice in short term experiments (less than 40 days). The findings of my study partially support those of Macedo *et al.* (1997), as the weight measurements were significant at the genotype and genotype x treatment levels. Aluminum concentration caused biomass to decrease, thus analysis of variance showed significant root, stem, and total biomass treatment effects (Table 14). However, in my study, the results of the stem-elongation data were just as useful, if not more so, than the biomass data in identifying genotype differences to Al. The results of this current study do support the conclusion of Macedo *et al.* (1997). Stem elongation data may be associated with a particular Al concentration; however, biomass was cumulative and does not allow determination of genotype threshold concentration.

Biomass may be correlated with Al tolerance as follows. Genotype 3D had the lowest total biomass, álbeit not significantly so, of the six genotypes in the Al treatments (Table 13). Low biomass, or a lack of growth, may explain why 3D had the highest concentration where the first significant decrease occurred in stem elongation, cumulative stem height, and stem count. One possible explanation of low biomass being related to Al tolerance may be that the resistance mechanism of the genotype is to grow slowly and thus limit Al toxicity. Genotype 3D produced the lowest amount of biomass in the study and endured the highest [Al]s, while 11T was affected in an opposite way.

In the Al treatments genotype 11T produced the largest amount of biomass in the study,

albeit not significantly greater than other genotypes, but was affected by the lowest concentrations in the study. Interestingly, genotype 11T did not produce significantly less biomass in Al treatments than in the control treatments. The plants could have been exporting Al to older leaves and dropping those leaves; the 11T genotype consistently had the lowest root, stem, and total biomass in controls. Because of the high concentration of Al endured before decreasing in growth rate and high biomass relative to other genotypes, the presumed tolerance of 11T may be a factor of variable growth. Genotype 11T may have begun to produce more biomass as a response to the [Al] increase. Stem biomass may be variable within genotypes due to variation in stem density (number of stems) or mortality and not due to ecotypic (genetic) differences.

The effect of high [Al] cancels out the effect of low [Al]; thus, Al may have caused an unidentified change in the biomass data because biomass was collected at the termination of the experiment. Because the biomass study was cumulative over the concentration range the cumulative effects should be analyzed as a relative rate or absolute data and not as a percentage of control. For example, stimulation of growth may have occurred at low concentrations in relation to control; however, by the end of the study the concentration was below control and the analysis of data described no noticeable effect (i.e. the stimulation increases in biomass of Vermillion (as evidenced by stem elongation) may have become obscured due to cumulative or additive results because of the subsequent decrease). Expressing the biomass data as percent of the control did not help to identify significant genotype effects due to the large variation in the data. Although genotype 11T would appear to be the most tolerant to Al (Table 13C), its total biomass as a percent of the control was not significantly different from the other genotypes (Table 15).

The biomass data (stem and total) allowed two groupings of tolerance: One grouping (primarly 11T) appeared to be the most tolerant because the Al treatment did not significantly reduce biomass relative to the 11T controls (Table 13C). Hence, Al concentration may appear to have a low level of toxicity on this genotype within the experimental Al concentration range. Aluminum toxicity of genotype 11T may be interpreted in two ways:

(A) The genotype 11T may have a genetically controlled low biomass production, even in the absence of Al. Hence, the effect of Al exposure is not obvious due to the low biomass production or slow growth of the genotype. The biomass of genotype 11T is sensitive to Al exposure; this conclusion agrees with stem elongation. The second grouping (all the genotypes except for the genotype 11T) all show reduced biomass with Al and none are significantly different from each other. Hence, biomass creates two groupings while stem elongation resulted in four groupings.

(B) Genotype 11T may be tolerant to Al because its biomass is not significantly affected by the Al treatment. If the genotype is biomass tolerant, the conclusion does not agree with the stem elongation data. I have insufficient data to determine which of these alternatives is valid.

5 Limitations of the Study

The results of this study were influenced by the environmental constraints of the growth chamber, the ability of roots to grow outside their containers, and a lack of information in the literature concerning the Al concentration range that negatively affects growth parameters of *Spartina alterniflora*. Roots and tillers were able to escape outside the containers and roots become intertwined with the roots of other specimens. The tips of the tallest leaves were scorched on the lamps. Unfortunately, the table could not be lowered to prevent scorching due to the sump location. A problem encountered in Al toxicity studies is selecting the appropriate Al concentrations so that the weakest genotypes in the study are affected as well as the most

resistant genotypes (Reid *et al.* 1971, Foy 1976, Macedo *et al.* 1997). This study would have been able to more accurately determine threshold level response and differences in Al tolerance among genotypes if pre-existing data were available on the toxicity range for this species. The following paragraphs discuss limitations concerning each of the major growth parameters used in this study.

a. Stem Elongation. Stem elongation, in contrast to cumulative stem height and stem count, was likely least affected by environmental limitations such as plant containers and the height of the growth chamber. The selection of young stems that were not so tall as to be burned for stem elongation alleviated the height restriction on this growth parameter. Stem elongation rate is not affected by sample size, or small initial variation in plant density, and provides the most usable data of all measurements taken due to it being a parameter relatively independent of specimen variation. Mendelssohn *et al*. (2001) similarly found that stem elongation was a reliable indication of photosynthetic rate.

b. Cumulative Stem Height. Rhizomes and roots were able to grow out the drainage holes in the containers and produce new shoots after only several weeks. The rhizomes intertwined, and it was difficult to know what genotype they were. Therefore, cumulative stem height was determined only on the stems growing inside the containers. Also, the environmental limitations of the plant growth chamber may have impaired the usefulness of the cumulative stem height data. Cumulative stem height was limited by the height between the table and the ceiling of the growth chamber. Leaf tips that came in contact with the Plexiglas® heat barrier of the growth chamber were burned from the heat of the lamps. Thus, the plants in the control treatment grew faster than the Al-dosed plants, and showed a decline in growth rate based on cumulative stem height, during the experiment. The leaves of the control group were substantially taller than those

of the Al experimental treatment. The tallest leaf tips of the control plants were unintentionally being burned on the growth chamber ceiling, [Al] effects may have been somewhat obscured or under estimated when the data was expressed as a percentage of the control. The burning of leaf tips may have caused the decreased relative growth rate in the control treatments of cumulative stem height and the number of stems. The experimental treatment did not lamp-burn similar to the lamp-burned control plants, and may be one of the reasons why there was little difference in the relative growth rate of cumulative stem height between control and experimental plants as was expected. The control plants became somewhat pot-bound over time as there was no more room for stems to emerge within the container; while the increasing [Al] was affecting the other treatment.

c. Stem Count. For the stem count live stems within the container were counted, thus stems escaping the container through rhizomes were not included. New tillers emerged from the drain holes in the plant container even though heavy cloth was put in the bottom to restrict root and rhizome penetration. After only several weeks, the roots intertwined with other specimens. When new stems emerged from the roots, it was unknown what genotype they were, thus, the stem counts were limited to the stems growing inside of the containers.

d. Biomass. Root material outside of the containers was not included in the results. The root material formed an intertwined mat that connected all of the plants in each of the treatments. The pot-bound conditions which occurred in this study exemplifies the importance of performing the experiment within an interval short enough to prevent the plants from becoming pot bound (Archambault *et al.* 1996). A shorter exposure interval or a better way to prevent roots and rhizomes from exiting the containers may allow for a more complete estimate of biomass.

e. Critical Toxicity Threshold. I was unable to determine the critical toxicity threshold for all

growth variables because the rates did not consistently decrease below 30 percent of controls. I did not dose in small enough [Al] intervals to separate genotypes based on the Thorton's critical toxicity threshold. Five of the six genotypes were affected by 5.4 mM Al. Before the decrease in growth began, the [Al] was 1.8 mM Al. Subsequent studies should utilize a narrower concentration dose interval at an arithmetic rate (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 mM) so that the critical toxicity threshold may be more precisely determined. A lesson learned in this study is that the [Al] interval must be narrow between 5.4 mM and 10.8mM and above to optimally gage genotype variation by using the critical toxicity level for stem elongation.

f. Percent Mortality. Leaf mortality or leaf browning occurred because of either, toxicity or natural leaf tissue senescence. Older leaves are commonly brown and dry and not any longer a living part of the plant. These brown leaves were selectively trimmed to reduce light interception. Also, when the leaves burned on the lamps, the brown material was removed. So that the strainer in the hydroponics pump would not clog, dead plant material was often removed and added to the final biomass measurements. Necrotic tissue was more plentiful in the Al experimental group. Thus percent mortality or the percent of brown material was always kept close to 10 to 30 percent throughout the experiment. Pictures of the plants show that after the full second week of 10.8 mM Al (18 weeks of Al exposure), there was no single plant that had 100 percent mortality (Figure 9). Thus, 100 percent mortality might either require a higher [Al], a longer exposure time, or both. In my study, all the plants still had green tissue and were apparently alive at the end of 18 weeks. Macedo *et al.* (1997) found that the necrosis criterion was a better indicator of toxicity than biomass parameters over long durations (longer than 80 days) at high [Al]. This study contradicts those of Macedo *et al.* (1997); although this may have been largely due to the trimming of necrotic material in the Al treatments. Macedo *et al.* (1997)

found that the use of a necrosis criterion may be the only reliable method to gauge Al toxicity in long term experiments at high concentrations of Al; my study does not support the conclusion that use of a necrosis criterion is a superior method for determining genotype variability of a single species.

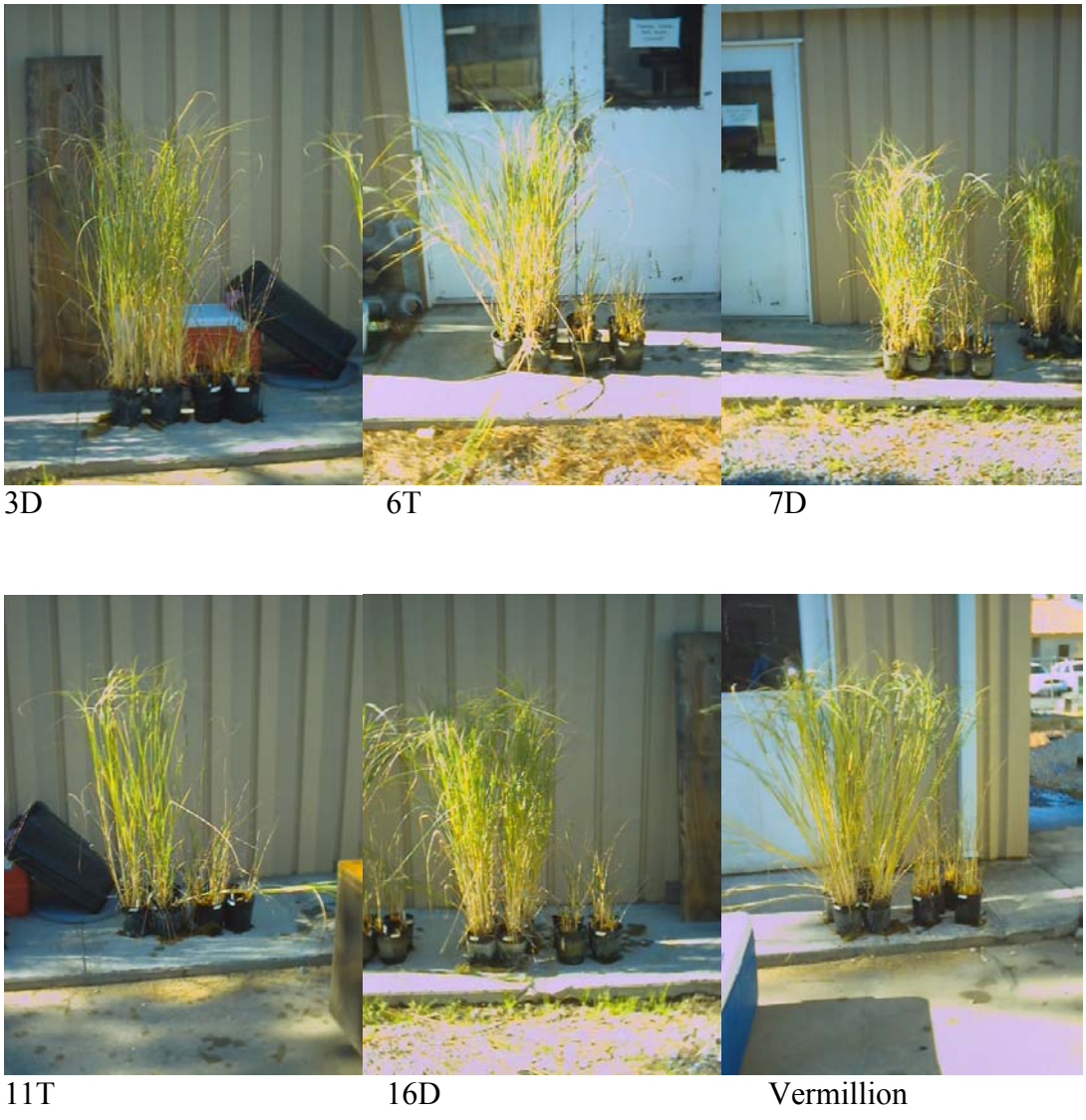


Figure 9. Six cultivars of *Spartina alterniflora* were grown for 16 weeks in increasing aluminum concentration. Four rows of three plants are shown, the two rows on the left are the control treatments and the two rows on the right are the experimental treatments.

6 Comparison of Al Toxicity for *Spartina alterniflora* with Other Species

The toxic [Al] for *Spartina alterniflora* was determined to be higher than any other species found in the literature. The literature pertains to upland rice, water spinach, and terrestrial species. Foy *et al.* (1978) describe how aluminum toxicity commonly resembles Ca deficiency. There is a common practice on arable land to apply gypsum ($CaSO_4$) or lime ($CaCO_{3)}$ to ameliorate the toxic effects of Al on plants. In the humid tropics the $CaCO_3$ content of soils is lower than in soils of arid and humid temperate regions (Prasittikhet and Gambrell 1989). Delhaize and Ryan (1995) found that Al-Ca interactions are the primary mechanism of Al toxicity. As a wetland plant was studied in my experiment, conditions for growth were optimal due to the hydroponics methods that circulated oxygenated nutrient solution through the pots. This likely prevented or at least reduced nutrient limitations at the root surface. Calcium was added to the hydroponic solution in both the nutrient solution and the synthetic sea salts. It is possible that the [Ca] could have imparted a resistance mechanism for preventing Al toxicity symptoms.

Schier (1996) determined whether differences occurred in threshold toxicity of new and one year old red spruce seedlings. The toxicity threshold for root dry weight was determined when the biomass of the experimental treatment became significantly less than control (0.06 mM Al). The needle dry weight and stem dry weight toxicity threshold was different for the age groups: 1 year old spruce was 0.4 mM Al while new seedlings were 0.8 mM Al. The toxicity threshold for plant height was approximately 0.4 mM Al.

Lidon and Barreiro (1998) developed dose response curves for maize to determine at which concentration a threshold toxicity may occur. The threshold toxicity appears to occur above 0.3 mM Al. Both root and shoot fresh weight and dry weight increased when

concentration was increased from 0 to 0.3 mM Al. The application of 0.9 mM Al caused all recorded weight measurements to decrease. The pH was 4.0 and the Al was added as [$Al_2(SO_4)_3$]. The researchers compared their dose-response curve with that of Ulrich (1952) and concluded that the threshold toxic tissue concentration was between 0 to 3.0 mM.

Sun and Wu (1998) determined the toxicity threshold concentration of water spinach. The plants were grown in cultures ranging 0 to 1.8 mM Al. The Spinach plants began to show symptoms of toxicity at 0.7 mM Al. Sun and Wu (1998) determined the toxicity threshold concentration of water spinach to be 1.8 mM Al.

Lux and Cumming (1999) found that the Thorton's critical toxicity level for tulip poplar seedlings was 0.190 mM Al (root was 0.512 mM Al). These concentrations reduced shoot and root biomass. The range where approximately 70 percent of damage occurred was between 0 and 0.2 mM Al. For tulip-poplar, the critical toxicity level was determined to be 0.2 mM Al (root was 0.5 mM Al). In my study, I found that the Thorton's critical toxicity level for *Spartina alterniflora* based on stem elongation was between 5.4 mM and 10.8 mM Al (depending on genotype); this threshold is greater than any other species found in the literature.

When a comparison of wetland and terrestrial species was made, Otte (2001) found that metal toxicity may not occur to wetland species. Metal toxicity to a terrestrial plant in a terrestrial environment must be distinguished from a wetland plant in a wetland environment, wetland plants have evolved mechanisms to tolerate metal stress beyond those mechanisms found in terrestrial plants. Wetland species normally endure more radical or different conditions than terrestrial plants. My research supports the finding of Otte (2001) that wetland plants do not undergo Al stress at the low metal concentrations that terrestrial plants do.

7 Conclusion

A prolonged drought event may have caused mortality to coastal wetlands. This study did not ascertain the cause for the brown-marsh event; however, it appears that [Al] would have to get very high for Al alone to have been the causative factor of brown-marsh. An [Al] increase alone does not explain the mortality of *Spartina alterniflora*. Toxicity, i.e. mortality, to *Spartina alterniflora* may occur if the [Al] increased over 10.8mM. The [Al] that causes mortality to *Spartina alterniflora* may be debatable. Macedo *et al.* (1997) reported that multiple growth parameters would be necessary to determine the relative Al toxicity between genotypes of the same species. In my study, stem elongation measurements resulted in significant genotype and concentration interactions. As [Al] increases the plant stops stem elongation, and the degree of decrease is genotype-specific. Aluminum significantly affects genotypes differently in stem elongation, cumulative stem height, and stem count. The plants would naturally exhibit mortality because old leaves die; although, I did not allow mortality to accumulate in this study due to selective trimming. The mortality of the whole plant would have occurred at higher [Al] than utilized in this study.

The null hypothesis that mortality of *Spartina alterniflora* to [Al] occurs outright was rejected. Similarly, the null hypothesis that Al does not have a toxic effect on *Spartina alterniflora* was rejected. The null hypothesis that there will be no significant difference among genotypes due to Al toxicity variation was rejected.

a. (A) What Is the Al Concentration that Exhibits a Toxic Effect on *Spartina alterniflora*?

The Al toxicity threshold concentration may be determined through the first significant decrease in growth rate when there is a significant genotype x concentration effect. For the genotypes, a significant reduction in stem elongation occurred at only 0.2 mM Al at the lowest

concentration and 10.8 mM Al at the highest concentration. For the species, it would appear that the critical Al concentration causing a growth reduction is between 0.2 and 10.8 mM, depending on genotype and parameter. The concentration causing complete plant mortality is greater than 10.8 mM Al.

Thorton's critical toxicity threshold for stem elongation was between 5.4 mM and 10.8 mM Al based on stem elongation rate expressed as a percent of the control. Using Thorton's critical toxicity threshold model, I was able to separate the genotypes into two groups. Using the first significant decrease in stem elongation rate approach for each genotype, the threshold was between 0.2 mM and 10.8 mM Al with genotypes falling into one of five groups.

Using the first significant decrease of cumulative stem height two general groupings may be made among the genotypes: sensitive and more resistant. Sensitive genotypes were affected by 5.4 mM and most resistant genotypes were affected by 10.8 mM Al. For the species, the [Al] that initiated leaf mortality based on stem count was 5.4 mM Al.

The first significant decrease based on stem count, was not applicable to stem count data. Three distinct groupings based on stem count may be made, those which decreased, those that increased, and those with no change. Increasing Al concentrations resulted in a stem count decrease in two genotypes, increase in one and three with no change. The [Al] that initiated stem mortality based on stem count was 10.8 mM Al.

b. (B) Is There Variability in Resistance to Al of the *Spartina alterniflora* Genotypes?

Based on the assumption that leaf elongation was the best indicator of growth response to Al, genotypes did vary in growth rates; although there was also variation in the other growth indicators, i.e., the ranking of the genotypes based on the first significant decrease results of the different parameters did not always correlate. Al toxicity threshold concentration may be

determined through the first significant decrease in growth rate if there is a significant genotype x concentration effect. The genotypes responded differently for each growth parameter measurement.

c. (C) Did Mortality or Growth Parameters Distinguish Genotype Resistance?

For the genotypes in the study the mortality was never 100 percent within the concentration range from 0.2 to 10.8 mM Al.; thus, mortality was not a reliable indicator of variation in Al tolerance among the genotypes. Growth parameters like stem elongation may have been the most reliable indicator of tolerance or resistance.

d. (D) Which Growth Parameter Most Accurately Describes Genotype Variation or Has the Best Resolution?

Using the first significant decrease approach when the genotype x concentration effect was significant ($p<0.05$) was effective for determining differences in genotypes. The growth parameters that were affected by [Al] varied with genotype (Table 17).

Table 17. The Al (mM) causing a significant decrease in stem elongation rate, cumulative stem height, and stem count for each of six genotypes of *Spartina alterniflora* and the three effects studied. A higher [Al] indicates a greater tolerance to Al.

	[Al] (mM)		
Genotype	Stem Elongation	Cumulative Stem Height	Stem Count
11T	0.6	10.8	no change †
16D	0.2	5.4	no change †
6T	10.8	5.4	increase
Vermillion	1.8	10.8 (2)	10.8 (2)
7D	5.4	10.8 (2)	10.8 (2)
3D	10.8	10.8 (2)	no change

(2) The second 10.8 dosage interval.
† Although there were some statistically significant increases and decreases, the highly variable data suggests indicates no biologically relevant differences with Al increase.

The first significant decrease in stem elongation rate, cumulative stem height, and stem count were more useful than critical toxicity level or dose response models. Mortality was

relatively constant due to selective trimming. Stem count did not always decrease. The most reliable indicator of toxicity was the first significant decrease approach in stem elongation and cumulative stem height. Biomass was only determined at the termination of the experiment. The biomass data provided inconclusive results when the analysis of variance was based on percent of control; however, the analysis of variance of the biomass data based on mass did denote a significant genotype effect related to genotype variability in Al toxicity.

e. 11T. Genotype 11T was intolerant to increasing Al. The toxic effect was noticed for stem elongation at 0.6 mM Al, and cumulative stem height at 10.8 mM Al. Stem count increased.

f. 16D. Of the six genotypes, 16D was Al intolerant and highly susceptible; the stem elongation rate of 16D was affected at 0.2 mM Al; the first significant decrease in cumulative stem height occurred at 5.4 mM Al. Stem count increased.

g. 6T. One of the most tolerant genotypes, 6T, had a stem elongation rate that was unaffected up to the first interval of 10.8 mM Al; although, cumulative stem height significantly decreased relative to control at 5.4 mM Al. Stem count increased.

h. Vermillion. Similar to 7D, Vermillion was moderately tolerant with stem elongation susceptible to only 1.8 mM Al; the cumulative stem height and stem count were affected by 10.8 mM Al (interval 2).

i. 7D. The genotype 7D was moderately Al tolerant. For 7D, stem elongation rate was significantly reduced at 5.4 mM Al, but cumulative stem height and stem count were affected by 10.8 mM Al.

j. 3D. The genotype 3D was highly tolerant to Al, enduring a concentration of 10.8 mM Al before a significant decline in elongation rate (at interval one), and cumulative stem height (at interval two); stem count remained unaffected by two intervals of 10.8 mM Al.

8 Suggestions for Future Research

The duration of the study must be sufficiently long to allow the concentration range to be increased until mortality is seen, but not so long that the plants over-grow the containers before the final dose. The mortality of the plants should occur before the highest dose is applied. Plant mortality may be a better indicator of genotype variability than growth parameters; although plant mortality did not occur within the [Al] range used in this study. The concentrations used should be consistently increased in arithmetic or logarithmic increments. The dose should be increased at each time interval to prevent plants from becoming acclimated to one concentration.

Cell culture techniques may be a quick and reliable alternative to the use of potted plants when determining differences in metal tolerance of plant genotypes. The use of cell culture technique has advantages not realized in growth chamber experiments. Genotypes have been selected for metal tolerance using cell culture (Yamamoto *et al.* 1996, Ishikawa and Wagatsuma 1998). Yamamoto *et al.* (1996) found that Al ions at pH 5 are a major growth limiting factor to cultured tobacco cells; also Al inhibits root growth within 1 to 2 hours. Ishikawa and Wagatsuma (1998) studied the effect of $AlCl_3$ on root tip cells after brief exposure of seedling roots to determine the plasma membrane permeability of root tip cells. He suggested that 0.5 hours exposure to the whole plant, or 10-minute exposure of protoplast, may be all that is needed to determine if a plant has reached a tolerance threshold for aluminum. The researchers suggest that similar technique may be used to determine tolerance variance in genotypes of a single species, and this method might be fruitful to pursue in wetland plants.

LITERATURE CITED

Adams, D.A. 1963. Factors influencing vascular plant zonation in North Carolina salt marshes. Ecology 44:445-456.

Alberts, J.J., Price, M.T., and Kania, M. 1990. Metal concentrations in tissues of *Spartina alterniflora* (Loisel.) and sediments of Georgia salt marshes. Estuarine, Coastal and Shelf Science 30:47-58.

Anderson, D.O., and Seip, H.M. 1999. Effects of a rainstorm high in sea-salts on labile inorganic aluminum in drainage from the acidified catchments of Lake Terjevann, southernmost Norway. Journal of Hydrology 224:64-79.

Archambault, D.J., Zhang, G., and Taylor, G.J. 1996. A comparison of the kinetics of aluminum (Al) uptake and distribution in roots of wheat (*Triticum aestivum*) using different aluminum sources. A revision of the operational definition of symplastic Al. Physiologia Planetarium 98:578-586.

Barceló, J., and Poschenrieder, C. 2002. Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminum toxicity and resistance: A Review. Environmental and Experimental Botany 48:75-92.

Brady, N.C., and Weil R.R. 1996. The Nature and Properties of Soils 11th edition. Prentice Hall, New Jersey 246 pp

Delhaize, E., and Ryan, P.R. 1995. Aluminum toxicity and tolerance in plants. Plant Physiology 107:315-321.

Delaune, R.D., Patrick, W.H., and Brannon, J.M. 1976. Coastal zone management series: Nutrient transformations in Louisiana salt marsh soils. Sea grant publication no.LSU-T-76-009

Foy, C.D., Chaney, R.L., and White, M.C. 1978. The physiology of metal toxicity. Annual Review of Plant Physiology 29:511-566.

Gallagher, J.L., Somers, G.F., Grant, D.M., and Seliskar, D.M. 1988 Persistent differences in two forms of *Spartina alterniflora*: a common garden experiment. Ecology 69:1005-1008.

Gambrell, R.P. 1994. Trace and toxic metals in wetlands-A Review. Journal of Environmental Quality 23:883-891.

Gambrell, R.P., Wiesepape, J. B., Patrick, W.H., Jr., and Duff, M.C. 1991. The effects of pH, redox and salinity on metal release form a contaminated sediment. Water Air and Soil Pollution 57-58:359-367.

Gutterridge, J.M.C., Quinlan, J.G., Clark, I, and Halliwell, B. !985. Aluminum salts accelerate peroxidation of membrane lipids stimulated by iron salts. Biochemica et Biophysica Acta 835:441-447.

Hoagland, D.R., and Arnon, D.I. 1950. The water culture method for growing plants without soil. Calif. Agric, Exp. Sta. Circular 347.

Howeler, R., and Cadavid, L.F. 1976. Screening of rice cultivars for tolerance to Al-toxicity in nutrient solutions as compared with a field screening method. Agronomy Journal 68:551-555.

International Rice Research Institute (IRRI) 1978. Annual Report for 1977. IRRI, Manila, Philippines.

Ishikawa, S., and Wagatsuma, J. 1998. Plasma membrane permeability of root tip cells following temporary exposure to Al ions is a rapid measure of Al tolerance among plant species. Plant Cell Physiol 39:516-525.

Kochian, L.V. 1995. Cellular mechanisms of aluminum toxicity and resistance in plants. Annual Review of Plant Physiology and Plant Molecular Biology 46:237-60.

Lidon, F.C., and Barreiro, M.G. 1998. Threshold aluminum toxicity in maize. Journal of Plant Nutrition 21:413-419.

Lux, H.B., and Cumming, J.R. 1999. Effect of aluminum on the growth and nutrition of tulip-poplar seedlings. Canadian Journal of Forestry Research 29:2003-2007.

Macedo de C.C., Kinet, J.M., and van Sint J.V. 1997. Effects of duration and intensity of aluminum stress on growth parameters in four rice genotypes differing in aluminum sensitivity. Journal of Plant Nutrition 20:181-193.

Mannahan S.E. 1994. Environmental Chemistry, In Chapter 3, “Fundamentals of Aquatic Chemistry”, Lewis Publishers, Boca Raton, Florida pp.45-86.

McKee, W.H., and McKevlin, M.R. 1993. Geochemical processes and nutrient uptake by plants in hydric soils. Environmental Toxicology and Chemistry 12:2197-2207.

McKee K.L., Mendelssohn I.A., and Materne, M.D. 2004. Acute salt marsh dieback in the Mississippi River deltaic plain: a drought-induced phenomenon? Global Ecology and Biogeography 13:65-73.

Mendelssohn, I.A., and Seneca, D.E. 1980. The influence of soil drainage on the growth of salt marsh cordgrass *Spartina alterniflora* in North Carolina. Marine Science 11:27-48.

Mendelssohn, I.A., and Postek, M.T. 1982. Elemental analysis of deposits on the roots of *Spartina alterniflora* Loisel. American Journal of Botany 69:904-912.

Mendelssohn, I.A., McKee, K.L., and Kong, T. 2001. A comparison of physiological indicators of sunblethal stress in wetland plants. Environmental and Experimental Botany 46:263-275.

Mooring, M.T., Cooper, A.W., and Seneca, E.D. 1971. Seed germination response and evidence for height ecophenes in *Spartina alterniflora* from North Carolina. American Journal of Botany 58:48-55.

Ono, K., Yamamoto, Y., Hachiya, A., and Matsumoto, H. 1995. Synergistic inhibition of growth by Aluminum and Iron of tobacco (*Nicotiana tabacumL.*) Cells in Suspension Culture. Plant Cell Physiology 36:115-125.

Otte M.L. 2001. What is stress to a wetland plant? Environmental and Experimental Botany 46:195-202.

Prasittikhet, J. and Gambrell, R. 1989. In Acid Sulfate Soils. In Acidic Precipitation, Vol. 4: Soils, Aquatic Processes, and Lake Acidification. Edited by Norton, S.A., Lindberg, S.E., and Page, A.L. Springler-Verlag, New York.

Rahman, R.M., Kawamura, K., Koyama, H., and Hara, T. 1998. Varietal differences in the growth of rice plants in response to aluminum and silicon. Soil Science and Plant Nutrition 44:423-431.

Reid, D.A., Fleming, A.L., and Foy, C.D. 1971. A method for determining Aluminum response of barley in nutrient solution in comparison to response in Al-toxic soil. Agronomy Journal 63:600-603.

SAS Institute 1998. SAS Users Guide: Statistics, Version 8.0 Edition. SAS Institute Inc., Cary N.C.

Schier, G.A. 1996. Effect of aluminum on growth of newly germinated and 1-year-old red spruce (*Pica rubens*) seedlngs. Canadian Journal of Forestry Research 26:1781-1787.

Schier, G.A., and McQuattie, C.J. 2000. Effect of water stress on aluminum toxicity in pitch pine seedlings. Journal of Plant Nutrition 23:637-647.

Sun, E.J., and Wu, F.Y. 1998. Along vein necrosis as indicator symptom on water spinach caused by nickle in water culture. Botanical Bulletin of the Academy of Singapore. 39:255-259.

Ulrich, A. 1952. Physiological bases for assessing the nutritional requirements of plants. Annu, Rev. Plant Physiol. 3:207-228.

Van Breeman, N., and Moorman, F.R. 1978. Iron-toxic soils. In: International Rice Research Institute. Soils and Rice. Los Baños, Phillippines 781-801 pp.

Wagatsuma, T., and Ezoe, Y. 1985. Effect of pH on ionic species of aluminum in medium and on aluminum toxicity under solution culture. Soil Science and Plant Nutrition 31:547-561.

Yamamoto, Y., Masamoto, M., Rikiishi, S., Hachiya, A., Yamaguchi, Y. and Matsumoto, H. 1996. Aluminum tolerance acquired during phosphate starvation in cultured tobacco cells. Plant Physiology 112:217-227.

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