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Effects of $CuCl_2$ on the Germination Response of Two Populations of the Saltmarsh Cordgrass, *Spartina alterniflora*

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The saltmarsh cordgrass (Spartina alterniflora) is the dominant vascular plant in tidal marshes along the east and gulf coasts of the United States. This plant's ability to survive in polluted estuaries has led many researchers to investigate its role in heavy metal uptake and export (e.g. Banus et al. 1974; Breteler et al. 1981; Giblin et al. 1983; Kraus et al. 1986).

Spartina seeds accumulate a variety of metals as well, although seed concentrations are generally lower than those found in leaf tissue (Kraus, 1988). It has been demonstrated that some heavy metals affect germination in this plant (e.g. methyl Hg, Zn, and Pb), while others, such as Cu and Cd, in solution concentrations as high as 100 mg/L, do not affect the germination response. Despite this, S. alterniflora seedlings grown in Cu solution exhibit 100 percent mortality within 56 d (Dunstan and Windom 1974).

MATERIALS AND METHODS

The present study was designed to examine population differences in the germination response of S. alterniflora to various concentration of Cu. Seeds collected at two different creek systems were used. One Creek, Sawmill Creek (SMC), is located in a polluted estuary in Lyndhurst, NJ USA (Kraus 1988). The control site, Big Sheepshead Creek (BSC), is a relatively non-polluted creek near Tuckerton, NJ USA (Kraus et al. 1986).

Soil samples, seeds, and leaves from both SMC and BSC were collected during the first week of October, 1986. The samples were gathered from several sites within both marsh systems. All samples were prepared for heavy metal analysis by digesting dry pulverized samples in analytical grade HNO_3 followed by H_2O_2. Digested samples were then refluxed in HCl (USEPA 1982). Samples were analyzed using atomic absorption spectrophotometry (Perkin-Elmer 272). Quality control was maintained by using standards of known metal content, as well as National Bureau of Standards River Sediment (NBS 1645) and Citrus Leaf (NBS 1572).

To ascertain whether population differences existed with respect to seed weight, seeds from both SMC (N=200) and BSC (N=200) were thoroughly cleaned in

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distilled water, then dried to a constant weight at 100 ^{o}C. Seeds were then weighed in groups of 100.

Prior to Cu treatment, field-collected seeds from both populations were stored under refrigeration in 40 g/L (4%) artificial seawater (ASW) (Instant Ocean). Seeds were held in 1 gallon plastic jars, in which a pin prick had been placed in the lid to facilitate water flow out of the jar as the seeds imbibed and swelled. Seeds were continuously immersed under these conditions for four months to facilitate stratification (Dr. E. Garbisch pers. comm.).

After stratification, seeds from both populations were placed in one of the following treatments: control (9.78% NaCl in distilled water), 25 mg/L $CuCl_2$ in 8.15% NaCl, 50 mg/L $CuCl_2$ in 6.53% NaCl solution, 100 mg/L $CuCl_2$ in 3.38% NaCl solution, or 150 mg/L $CuCl_2$ in distilled water. Salinity of solutions was equivalent in each treatment. During the experiments, seeds were maintained in plastic petri dishes. Seeds were placed on filter paper and treated with 5ml of the appropriate solution. Distilled water was added as needed to maintain the appropriate dillutions. Seeds were placed twenty (20) to a petri dish, for a total of 240 seeds from each population in each treatment. All treated seeds were placed in a temperature controlled (27 ^{o}C) growth chamber with a 12 hour light/12 hour dark cycle. Experiments were run for 18 d.

Epicotyl and radicle lengths of each germinated seed were measured at the end of the experiment. These data were analyzed using the Students t-Test, and Chi-Square.

RESULTS AND DISCUSSION

In this study, calculated Cu content of the NBS standards agreed closely with the certified values. Average amounts of Cu in SMC soil, leaf, and seed samples were 164.5 mg/kg, 45.3 mg/kg, and 37.1 mg/kg respectively. BSC values were 42.6 mg/kg (soil), 13.0 mg/kg (leaf), and 5.4 mg/kg (seed).

Average seed weights (dry wt) for BSC and SMC were 1.9 mg/seed and 2.0 mg/seed, respectively. Germination rates in the BSC control group were significantly greater ($P<0.05$) than in the 50 mg/L treatments. No significant difference existed between the BSC control and the 25, 100 or 150 mg/L treatments with respect to germination (Figure 1). Although no significant difference in germination rates existed between the BSC and SMC control groups, the SMC control group had a significantly greater ($P<0.05$) germination rate than did any of the SMC treatments (Figure 1).

All germinated seeds in both groups exhibited epicotyl growth, although radicles did not always emerge. There was no significant difference in epicotyl lengths in the BSC treatments after 18 d. Significant differences ($P<0.05$) between the SMC control group and the 50 mg/L treatment existed, however. A trend towards greater epicotyl growth ($0.1 > P > 0.05$) in the SMC Cu treatments was also noted when compared to the SMC control group (Figure 2).Radicles developed in 89% of the germinated BSC control seeds (33% of all control seeds). Only 54% of the 25 mg/L BSC treated seeds which exhibited epicotyl emergence also produced radicles (14% of all seeds in this treatment). No radicle emergence was noted in the other BSC treatments. These data were significant at the 0.05 level. Only 1% of the SMC germinated control seeds produced radicles. No radicles emerged in the other SMC treatments (Figure 3).

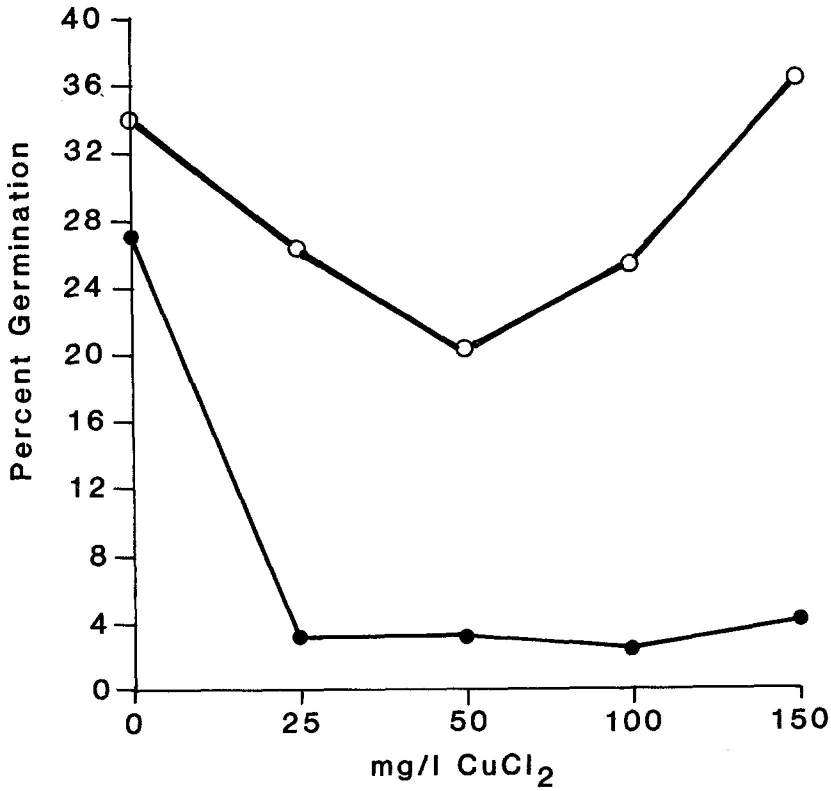

Figure 1 - Percent germination of stratified Spartina alterniflora seeds from SMC and BSC in control and copper treatments. O BSC ● SMC

S. alterniflora seeds from both populations germinated equally as well in the control treatments, but SMC seeds produced shorter epicotyls, and fewer radicles than did BSC seeds. Since seeds were collected over a wide area of both marsh systems, this phenomenon does not represent an accidental collection of low viability seeds at SMC from a single, localized spot. These data suggest that the SMC plants, which are from a heavily polluted marsh, may depend predominantly on vegetative reproduction for population maintenance. In a highly stressed system, those organisms which are successfully "coping" will out compete conspecifics which are not as well adapted. Therefore, asexual clones of successful parents should be as successful in the same environment. The genetic variability inherent in sexual reproduction may actually be less desirable under the stressed conditions found at Sawmill Creek.

Both populations of S. alterniflora exerted comparable amounts of energy in seed production, as evidenced by equivalent seed weights. Despite this, SMC seeds did not exhibit as robust of a germination as did BSC seeds. SMC control seeds produced significantly shorter epicotyls, and fewer radicles emerged than in the BSC control group. This could be attributed to Cu inhibition of germination in SMC seeds due to their relatively high Cu content at the start of the experiments

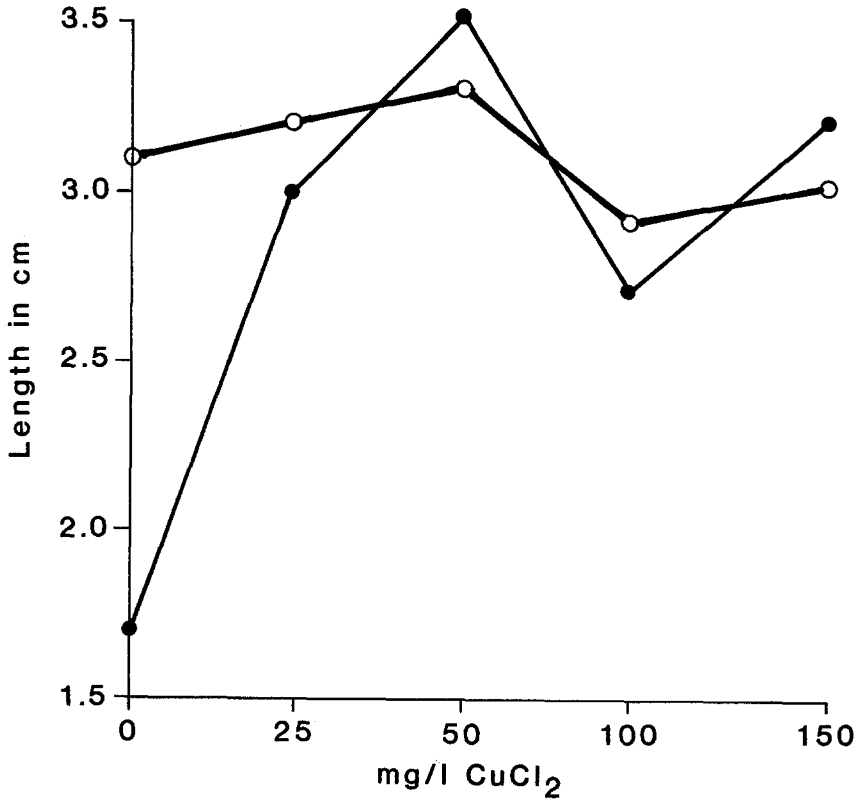

Figure 2 - Length of epicotyls from control and copper treated seeds collected from SMC and BSC. Data are reported as total average epicotyl length after the 18-day experimental period. O BSC ● SMC

(37.1 mg/kg), as compared to the BSC group (5.4 mg/kg), or possibly to low genetic diversity in the SMC population, which could, in effect, produce low viability seed stock.

Both populations exhibited interesting responses to the copper treatments. The BSC population exhibited a decrease in germination rates in the 50 mg/L Cu treatments when compared to the control group, but showed no difference in the 25, 100 and 150 mg/L treatments when compared to the control. At the same time, epicotyl length remained fairly constant throughout the treatments. BSC radicle emergence exhibited a different trend. Almost all of the BSC control seeds that germinated produced radicles, while only half of the BSC seeds germinating in the 25 mg/L treatment produced radicles. All of the other copper treatments completely inhibited radicle emergence. SMC germination rates exhibited a more typical pattern. Substantially fewer seeds germinated in the copper treatments than in the control. Despite this, epicotyl lengths from SMC seeds germinated in $CuCl_2$ were longer than the controls. Interestingly, only SMC control seeds produced radicles.

The heightened germination of BSC seeds, and the elongation of SMC epicotyls in the higher copper concentrations, is probably due to physiological responses to

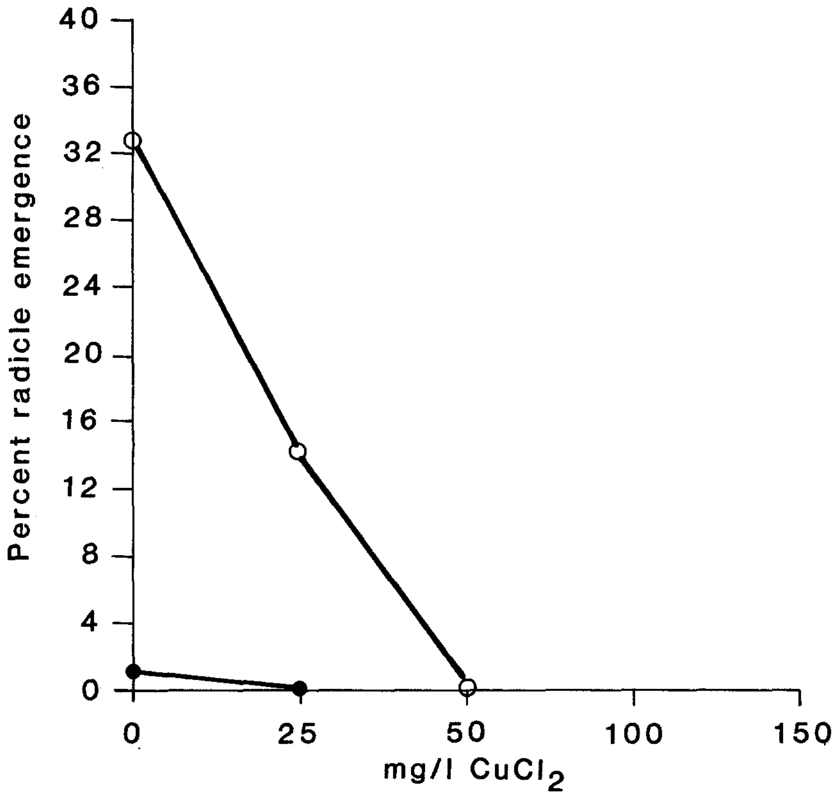

Figure 3 - Percent radicle emergence for control and copper treated seeds from SMC and BSC. Data are reported for all radicle emergence over the 18-day experimental period. O BSC ● SMC

copper stress. It is possible that the lower concentration of copper inhibited germination and growth, but higher copper concentrations stimulated the seedlings, and caused them to over compensate due to the stress. It should also be noted that in the copper treatments less energy was expended towards root tissue production, making more energy available for shoot production. At this point it is not clear whether copper actually inhibits root growth, or whether, under copper stress, more energy is expended in shoot growth. This could possibly be beneficial in that shoot growth would provide extra energy through photosynthesis, which in turn may be needed to compensate for the copper stress.

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