Limnol. Oceanogr., 35(2), 1990, 399–408

Mechanism for the hydrogen sulfide-induced growth limitation in wetland macrophytes

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Abstract

Hydrogen sulfide, a phytotoxin that often accumulates in anoxic marine and freshwater marsh soils, suppressed the activity of alcohol dehydrogenase (ADH), the enzyme that catalyzes the terminal step in alcoholic fermentation, in the roots of two wetland macrophytes. This inhibition of root ADH activity with increasing sulfide concentration was associated with decreases in root total adenine nucleotide pool (ATP + ADP + AMP), the adenylate energy charge ratio (AEC), nitrogen uptake (percent recovery of $^{15}NH_4^+$-N) and growth (leaf elongation). These responses were species-specific with a greater negative impact in the freshwater marsh species that naturally inhabits low-sulfide environments. These findings lend support to the hypotheses that ADH activity, as a measure of fermentative metabolism, is important in maintaining the root energy status of wetland plants under hypoxic-anoxic conditions, that there is a significant negative effect of H_2S on the anoxic production of energy in these roots, and that an important negative effect of H_2S on plant growth is an inhibition of the energy-dependent process of N uptake.

Wetland macrophytes, including *Spartina alterniflora,* the dominant salt-marsh species in North America, are characterized by high rates of primary productivity. The importance of this carbon source to the energy base of coastal and inland fisheries production has stimulated considerable research on the factors controlling plant growth and primary production in wetlands. Factors most frequently cited as important in influencing marsh plant growth include salinity (Nestler 1977), tidal inundation (Odum and Fanning 1973), soil waterlogging (e.g. Linthurst and Seneca 1980; Howes et al. 1981), and nutrient deficiencies (Mendelssohn 1979*a,b*).

Fertilization experiments have led to the consensus that N is the primary nutrient limiting salt-marsh primary production (e.g. Sullivan and Daiber 1974; Valiela and Teal 1974). Some investigators have suggested that growth limitation of *S. alterniflora* may occur through an inhibition of NH_4^+ uptake by some factor(s) associated with soil waterlogging (Mendelssohn and Seneca 1980; Morris 1980). This hypothesis is supported by the finding that the shorter, less productive form of *S. alterniflora,* which often inhabits more waterlogged regions of the marsh, is limited (Mendelssohn 1979*a,b*), yet the bulk soil contains higher concentrations of NH_4^+ than is found in more productive areas (e.g. streamside or tall form) (Mendelssohn 1979*b*; Delaune et al. 1983). In addition, half-saturation constants for NH_4^+ uptake are too low to explain the N limitation found in *S. alterniflora* marshes (Morris 1980).

Soil waterlogging may influence the interactions of soils and roots in several ways. Saturation of a soil with water reduces the rate of oxygen diffusion (Gambrell and Patrick 1978) and thereby limits the amount of oxygen available for plant root and microbial respiration. Wetland plants must not only adapt to root anaerobiosis, but must also contend with the edaphic microbial production of phytotoxins. When a soil is flooded and oxygen becomes limiting, heterotrophic facultatively and obligately anaerobic microorganisms in the soil use inorganic ions as terminal electron acceptors to break down organic matter (Turner and Patrick 1968). The flooded soil is thereby

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Acknowledgments

We thank D. M. Burdick, R. E. Turner, and R. Twilley for reviewing the manuscript and K. Flynn and J. Chupasko for technical assistance.

This work was supported by funds from the Louisiana Sea Grant Program (National Oceanic and Atmospheric Administration of the U.S. Department of Commerce).

rapidly converted through microbial respiration to a biochemically reduced state (Turner and Patrick 1968).

Hydrogen sulfide—a known phytotoxin to wetland plants (Okajima and Takagi 1955; Goodman and Williams 1961; Koch and Mendelssohn 1989)—may accumulate with persistent soil waterlogging in natural marshes due to the biochemical reduction of sulfate to sulfide by dissimilatory SO_4^{2-}-reducing bacteria (e.g. *Desulfovibrio*) (Postgate 1959). Concentrations of sulfide in marine soils have been found to exceed 1 mM (e.g. Carlson and Forrest 1982; King et al. 1982; Howes et al. 1985). This phytotoxin has been shown to reduce growth in several marine and freshwater marsh species (Joshi et al. 1975; Ingold and Havill 1984), but the underlying physiological mechanism by which this growth inhibition occurs, particularly in wetland plants, is not well understood.

We present data supporting the supposition that the growth-limiting effect of H_2S on plants inhabiting waterlogged soil environments results from an inhibition of the anoxic generation of energy via alcoholic fermentation and a concomitant reduction in plant N uptake. This study investigates the effects of aeration, hypoxia, and hypoxia plus sulfide on root ADH activity, root energy status, nitrogen uptake, and leaf growth of two wetland species: *S. alterniflora* Loisel. (salt-marsh species) and *Panicum hemitomon* Schultes (freshwater marsh species). These two macrophytes were chosen in order to compare a salt-marsh species that in nature is frequently exposed to high sulfide concentrations (>1.0 mM; Mendelssohn and McKee 1988) to a freshwater marsh species that rarely comes in contact with high sulfide levels (<0.1 mM; McKee and Mendelssohn unpubl. data).

Methods

Plant source and pretreatment—Monospecific stands of *S. alterniflora* from a salt marsh (29°20′N, 90°40′W) and *P. hemitomon* from a freshwater marsh (30°0′N, 90°40′W) were sampled in Terrebonne Parish, Louisiana. Several plugs with intact vegetation (15 × 50 cm) were extracted from the marsh and transported to the greenhouse. Stems were separated carefully to avoid injury to roots and rhizomes. Each plant was placed in a small plastic cup filled with sand and watered with full-strength Hoaglands + Fe-EDTA nutrient solution until enough root material was present to conduct the experiment (2 weeks). Thirty-five *S. alterniflora* plants with similar heights (~10–15 cm) were chosen for seven treatments ($n = 5$). Fifty *P. hemitomon* plants were selected for five treatments, but with two plants (20–25 cm high) per experimental vessel ($n = 5$). The number of *P. hemitomon* plants, which produce relatively smaller amounts of roots, was doubled to ensure enough tissue for biochemical analysis. Plants were carefully removed from the plastic cups and the sand gently washed off before transfer to the experimental vessels.

Experimental design—The experiments were conducted in sealed hydroponic culture solution with sulfide concentrations of 0.5, 1.0, 2.0, 3.0, and 4.0 mM for *S. alterniflora* and 0.5, 1.0, and 2.0 mM for *P. hemitomon*. In addition, a hypoxic treatment (0.0 mM sulfide level) was used to test for the effect of low oxygen tension alone. Well-aerated plants in buffered culture solution served as controls. The experiments were conducted (September 1988) as a completely random design ($n = 5$). Nutrient solution (full-strength Hoaglands + Fe-EDTA) containing MES buffer (pH 7.0) and a nitrification inhibitor (N-Serve) was bubbled with N_2 gas for 45 min and added to Erlenmeyer flasks (250 ml) that were stopped immediately. Plants were previously inserted through holes in the stoppers and positioned in the flasks to totally immerse the roots in the nutrient solution. Flasks were then sealed with a nontoxic silicone and wrapped in aluminum foil to exclude light from the roots.

The aerated treatment for both species was achieved by bubbling the media continuously with air through a 0.1-cm-diameter tube. The hypoxic treatment consisted of deoxygenated nutrient solution created by bubbling the culture media with N_2 gas for 45 min before introducing the plants. The hypoxic sulfide treatments were achieved by adding sulfide ($Na_2S \cdot 9H_2O$) to the deoxygenated nutrient buffer solution. After sulfide was added at the start of the

experiment, $^{15}NH_4^+$ (99.0 atom% excess) was added via syringe to achieve a final concentration of 5.0 ppm (357 μM) in the flask.

A 5-ml portion of the medium from each flask was extracted daily by syringe and immediately injected into an equal volume of antioxidant buffer (Lazar operating instructions for model IS-146 sulfide electrode). Sulfide (S^{2-}) concentration was then measured with a selective ion electrode (Lazar Corp.). A standard curve was calculated from a series of dilutions of Na_2S prepared with antioxidant buffer. Concentrations of S^{2-} in the experimental vessels were thus adjusted to the appropriate treatment level and maintained at the levels shown in Table 1. If no adjustment was necessary, 5.0 ml of deionized, deoxygenated water was injected into the flask to keep all water volumes equal. The two experiments were conducted sequentially due to the time-consuming process of measuring and maintaining sulfide concentrations in each vessel daily. The experimental period for *S. alterniflora* (6 d) was longer than for *P. hemitomon* (2 d) because of the rapid appearance of lethal symptoms (i.e. leaf curling and yellowing) in *P. hemitomon.*

Growth measurements—With a leaf-marking technique, leaf elongation was measured as an index of plant growth. A nonreactive silicone dot placed at the base of the newest leaf blade provided a means of following leaf expansion relative to a fixed point. The expansion of the newly developing leaf (distance between marker dot on leaf and stem) was recorded daily throughout the experiment.

Solution redox potential—At the end of each experiment, plants and stoppers were removed from the flasks and redox potentials (Eh) measured. Brightened Pt electrodes (n = 3), previously checked with quinhydrone in pH buffer 4.0 (218 mV) and 7.0 (40.8 mV), were inserted with a calomel reference electrode into the experimental media. Eh values were corrected by adding the potential of the calomel reference electrode (244 mV) to the millivolt reading.

Root extractions and assays—Turgid, structurally intact roots with an apparent white stele were frozen with liquid nitrogen upon removal from the culture solution.

Table 1. Average sulfide concentrations (mM) achieved in the treatment flasks during the 6-d (*Spartina alterniflora*) and 2-d (*Panicum hemitomon*) experiments. Sulfide concentrations measured in aerated and nonaerated (0.0 mM) flasks were below detection limits (<0.01 mM). Values represent the mean±SE of concentrations measured 10 times for *S. alterniflora* and 3 times for *P. hemitomon* during the two experimental periods; n = 50 and 15, respectively.

Treatment (sulfide level)	*S. alterniflora*	*P. hemitomon*
Nonaerated (0.5 mM)	0.36±0.02	0.39±0.08
Nonaerated (1.0 mM)	0.75±0.05	0.76±0.14
Nonaerated (2.0 mM)	1.55±0.10	1.90±0.35
Nonaerated (3.0 mM)	2.64±0.16	—
Nonaerated (4.0 mM)	3.73±0.26	—

Roots were lyophilized, weighed, and ground in a Wiley mill (60-mesh sieve).

Adenine nucleotide concentrations—Tissue (0.05 g) for the determination of adenine nucleotides and the energy charge ratio, AEC = [ATP + 0.5 ADP] : [ATP + ADP + AMP] (Atkinson 1968), was extracted in 10 ml of boiling 1 mM EDTA with 5% (wt/vol) polyvinylpolypyrrolidone (PVPP) at pH 7.4 for 30 s. The extraction solution was centrifuged at 4°C and 20,000 × g for 15 min and assayed according to Mendelssohn and McKee (1981). ATP was measured with an ATP photometer (Analytical Development Co.) and the firefly luciferin-luciferase (FLE-50, Sigma Chemical Co.) complex. ATP was measured directly; ADP and AMP were converted enzymatically to ATP and determined by subtraction. Percent recoveries of internal ATP, ADP, and AMP standards were never <80% and were unaffected by treatments.

Alcohol dehydrogenase activity—Tissue for ADH analysis was extracted by vortexing 0.025 g in 5 ml of 17 mM HEPES buffer (pH 8.0) with PVPP (5%) for 30 s. The extract was centrifuged at 4°C and 20,000 × g for 15 min. The assay was conducted at 30°C in 5.4 mM $MgCl_2$, 0.26 mM NADH, and 0.40 mM acetaldehyde in 14 mM Tris buffer (pH 8.0) in a total volume of 2.8 ml. Enzyme activity, assayed spectrophotometrically by following the oxidation of NADH at 340 nm, remained linear in all cases for 4–5 min. Recoveries of internal standard ADH were high (>85%) and indicated no differential inhibition due to species or treatment during analysis.

Nitrogen isotope analysis—Plant tissue was ground as described above and analyzed for nitrogen content (TKN) by a semi-micro-Kjeldahl method (Buresh et al. 1982). The samples were oxidized with NaOBr, and ^{15}N content was determined with a Dupont 21–614 isotope ratio mass spectrometer according to Hauck (1982). Atom% ^{15}N was determined with the ratio of the 28 (^{14}N:^{14}N) and 29 (^{14}N:^{15}N) peak heights, as well as the 30 (^{15}N:^{15}N) peak heights when atom% ^{15}N was >5.0. Atom% ^{15}N excess was determined by subtracting the atom% ^{15}N of unlabeled NH_4^+ (0.349) from the calculated atom% ^{15}N of each sample. This excess was multiplied by the total weight of N in the plant tissue to determine atom% ^{15}N tissue recovery. Total percent recovery in the root and leaf was determined by dividing ^{15}N in the tissue by that added to the culture media and multiplying by 100.

Statistical analysis—Analysis of variance (ANOVA; proc GLM), regression (proc REG), and contrast (proc GLM) were performed with the SAS statistical package (SAS 1982). Statistically significant differences between treatment means were found with Duncan's multiple range test (ANOVA). All means are significantly different at the 0.05 level unless otherwise stated.

Results

Redox potential—Redox values were high in the aerated treatment (+400 to +700 mV), but significantly lower (0 to +125 mV) in the treatment that was initially bubbled with N_2 gas (Fig. 1a). An increase in sulfide concentration in the hypoxic culture solution from 0.0 to 4.0 mM for *S. alterniflora* and from 0.0 to 2.0 mM for *P. hemitomon* resulted in a linear decrease in Eh from +60 to −170 mV ($R^2 = 0.84$) and from +93 to −100 mV ($R^2 = 0.87$) (Fig. 1a).

Plant growth—Leaf elongation was significantly slower in the anoxic treatment (0.0 mM sulfide) compared with the aerated treatment for both species (Fig. 1d). Leaf elongation was further reduced by adding sulfide to the culture media. The freshwater marsh species, *P. hemitomon,* however, was significantly affected at a lower concentration of sulfide (1.0 mM) than was the salt marsh species, *S. alterniflora* (2.0 mM). These sulfide treatments reduced average leaf growth in *S. alterniflora* and *P. hemitomon* by 42 and 200% (Fig. 1d).

ADH activity—Under hypoxic conditions, ADH activity was increased fourfold in *S. alterniflora* and twofold in *P. hemitomon* roots compared to the aerated treatment which exhibited limited ADH activity (Fig. 1b). At redox potentials of +60 mV for *S. alterniflora* and +93 mV in *P. hemitomon,* root ADH activity was stimulated (Fig. 1a,b). Sulfide concentrations of 1.0 mM in *P. hemitomon* and 2.0 mM in *S. alterniflora* culture solution resulted, however, in significant inhibition ($P < 0.05$) of root ADH activity. This inhibition occurred even though addition of sulfide caused the Eh of the culture solution to decline (Fig. 1a,b). With an increase in sulfide level from 0.0 to 4.0 mM, *S. alterniflora* root ADH activity decreased (slope = −0.46; $P < 0.01$) after 6 d. Although ADH activity was significantly inhibited in *P. hemitomon* roots exposed to 1.0 and 2.0 mM sulfide, it was higher at 0.5 mM sulfide relative to the nonaerated (0.0 mM sulfide) treatment. The shorter exposure time for *P. hemitomon* (2 d) may be the reason for the lack of inhibition of ADH activity at 0.5 mM sulfide compared to *S. alterniflora* (6 d).

Adenine nucleotides and AEC ratio—Total adenine nucleotide concentrations decreased in both species when ADH activities increased in the hypoxic roots (Fig. 1c), but the AEC ratio—an indicator of metabolic activity describing the equilibrium between ATP-generating and -utilizing reactions (Atkinson 1968)—was maintained during root hypoxia in *S. alterniflora.* Although *P. hemitomon* root AEC ratio was lower under hypoxic conditions, multiple range testing showed no significant difference between the aerated and nonaerated (0.0 mM sulfide) treatment.

Neither species was able to maintain its total adenylate pool or AEC ratio when sulfide was present in the culture media (Fig. 1c). After 2 d of exposure to sulfide treatments, *P. hemitomon* showed a significant reduction in total adenylate concentrations and AEC at sulfide concentrations of 1.0 mM and above (Fig. 1c). *Spartina alterniflora* also exhibited significantly reduced ad-

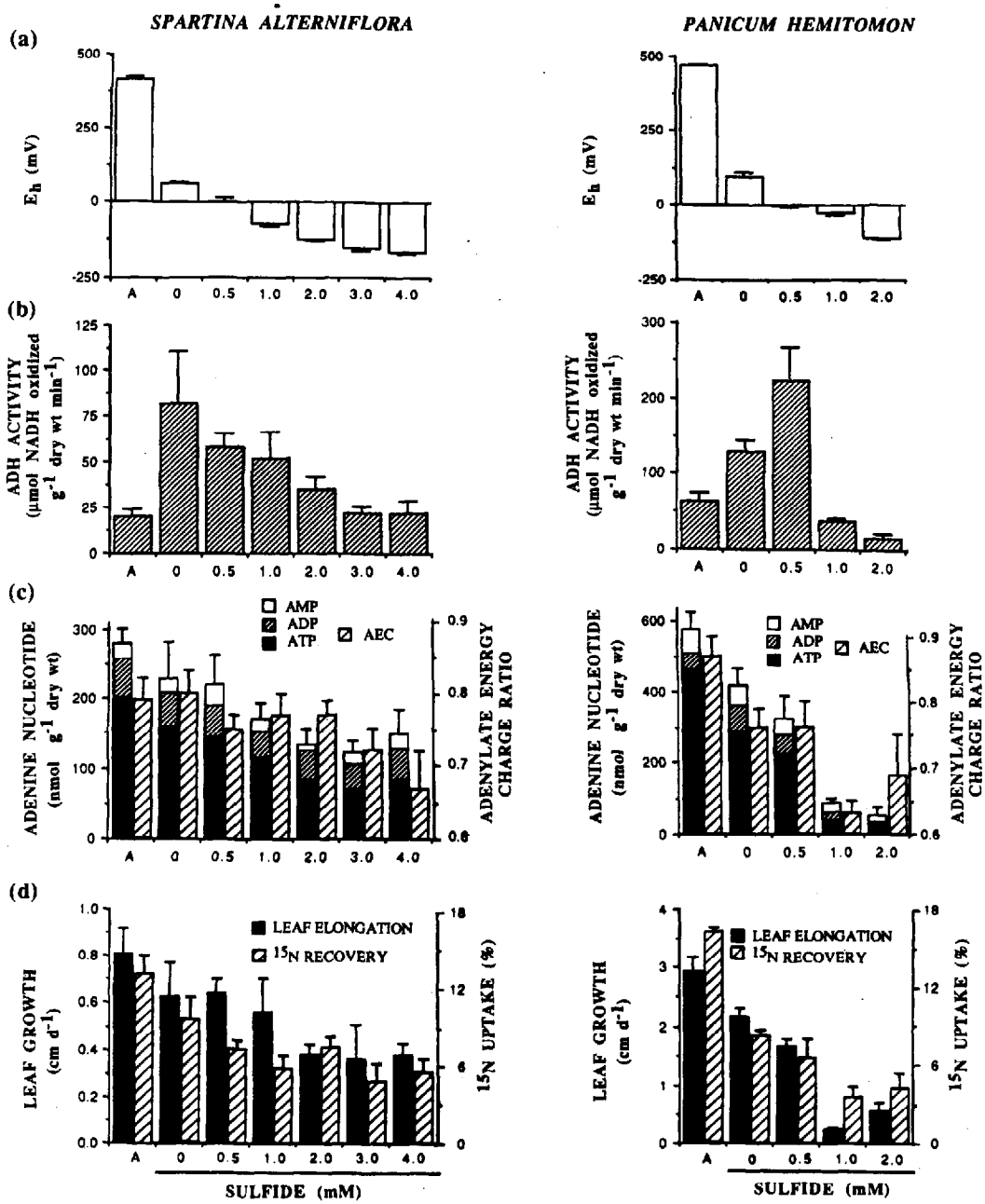

Fig. 1. *Spartina alterniflora* culture solution experiment (6 d) in response to the seven treatments applied [aerated (A), hypoxic (0.0 mM sulfide), and hypoxic with sulfide additions of 0.5, 1.0, 2.0, 3.0, and 4.0 mM] and *P. hemitomon* experiment (2 d) with five treatments applied [aerated (A), hypoxic (0.0 mM sulfide), and hypoxic with sulfide additions of 0.5, 1.0, and 2.0 mM]. a. Changes in oxidation–reduction potentials (Eh) in the culture solutions. b. Alcohol dehydrogenase activity (ADH) in the roots. c. Total adenine nucleotide pool (ATP + ADP + AMP) and adenylate energy charge ratio (AEC) in the roots. d. Daily leaf elongation rates, averaged over the experimental period and total $^{15}NH_4^+$-N percent recovery in the roots. Error bars represent the standard errors of the means (n = 5).

Table 2. Leaf $^{15}NH_4^+$ recovery in *Spartina alterniflora* and *Panicum hemitomon*. Values represent the mean ± SE (n = 5). Asterisk: significantly different means ($P < 0.05$; Duncan's multiple range test).

Treatment	[Sulfide] (mM)	Leaf ^{15}N recovery (%)	
		S. alterniflora	*P. hemitomon*
Aerated	0.0	27.17±3.00	28.28±8.14
Nonaerated	0.0	28.31±3.19	26.19±2.77
Nonaerated	0.5*	25.40±4.71	12.82±3.49
Nonaerated	1.0*	32.32±3.57	6.61±1.47
Nonaerated	2.0*	26.51±4.04	11.15±1.75
Nonaerated	3.0	22.79±7.14	—
Nonaerated	4.0	30.84±7.30	—

enylate concentrations and AEC, but at higher (3.0 and 4.0 mM) sulfide levels (Fig. 1c).

Recovery of ^{15}N in root and leaf tissue—N uptake in the roots—a metabolic function dependent on energy production—was found to be limited by the hypoxic and sulfide treatments in *S. alterniflora* and *P. hemitomon* ($P < 0.01$). Hypoxic conditions in the culture solution containing 357 μM $^{15}NH_4^+$ significantly reduced the total ^{15}N% recovery in the roots of *S. alterniflora* (5%) and *P. hemitomon* (7%) compared to the aerated roots (Fig. 1d). Multiple range tests showed a further reduction of the percent ^{15}N recovery in the roots at sulfide levels ≥3.0 mM in *S. alterniflora* (4–5%) and ≥1.0 mM in *P. hemitomon* (3–5%) relative to unaerated roots without sulfide exposure (Fig. 1d). These results are supported by linear contrast analysis that showed a highly significant difference ($P < 0.01$) in root ^{15}N recovery between unaerated controls and sulfide-amended plants of both species. Recovery of *S. alterniflora* leaf ^{15}N was not significantly different as a function of treatment, but differences were highly significant ($P < 0.01$) among the aerated, hypoxic, and sulfide-amended *P. hemitomon* plants (Table 2).

Discussion

When drained soils are flooded, oxygen diffusion is reduced 10,000 times (Gambrell and Patrick 1978). This slow rate of diffusion combined with the respiratory demands of plant roots and soil microorganisms results in little or no oxygen available externally to plant roots and the accumulation of reduced inorganic and organic compounds in the soil. The redox potential (Eh), which is a measure of the intensity of soil reduction, decreases from a highly oxidized state (+700 mV) when the soil is drained to a moderately (+100 mV) or strongly reduced state (−300 mV) when flooded. In the aerated culture solution, the Eh for both species (Fig. 1a) was high and occurred within the range (+400 to +700 mV) characteristic of a well-drained soil where oxygen is available and utilized as the preferred electron acceptor (Turner and Patrick 1968). Bubbling the culture solution with N_2 gas resulted in a significantly lower redox status that was indicative of anoxic conditions (Fig. 1a). In the natural environment, sulfide accumulates in strongly reducing soil conditions where sulfate is available (e.g. through inputs of seawater) for reduction by bacteria (Postgate 1959). The addition of Na_2S to the culture solution successfully produced a range of sulfide treatment levels (Table 1) similar to that encountered in salt marshes (Delaune et al. 1983; Howes et al. 1985; Mendelssohn and McKee 1988; McKee and Mendelssohn unpubl. data). The increase in sulfide concentration resulted in a linear decrease in Eh (Fig. 1a). Similar negative relationships between sulfide concentrations and redox potentials have been found in wetland soils in the field (Delaune et al. 1983; Mendelssohn and McKee 1988) and in laboratory soil suspension experiments (Connell and Patrick 1968).

Plants exposed to an anoxic root environment must have a means for internal oxygen transport if they are to maintain aerobic root respiration. Although most wetland plants adapted to anoxic conditions possess aerenchyma or airspace tissue (Armstrong 1979), this mechanism is not always sufficient to maintain completely aerobic metabolism in the roots (*see* Crawford 1982; Mendelssohn and Burdick 1988). Even a species with an extensive aerenchyma system such as *S. alterniflora* may suffer root oxygen deficiencies when exposed to extremely waterlogged conditions (Mendelssohn et al. 1981). Root-cell hypoxia can result in a switch to anoxic metabolism as indicated by an increase in the activity of

alcohol dehydrogenase (ADH)—the enzyme catalyzing the terminal step in alcoholic fermentation (Mendelssohn and Burdick 1988). *Spartina alterniflora* has been found to increase root ADH activity in response to a hypoxic root environment in the laboratory (Mendelssohn and McKee 1987), as well as to soil waterlogging in the field (Mendelssohn et al. 1981; Mendelssohn and McKee 1988). Similarly, we found ADH activity to be stimulated fourfold in *S. alterniflora* roots and twofold in *P. hemitomon* roots in the hypoxic treatment compared to the aerated treatment, which exhibited limited ADH activity (Fig. 1b). This response indicated a root oxygen deficiency in plants grown in the hypoxic media and the potential for alcoholic fermentation (Smith and ap Rees 1979; Keeley 1979; Perata et al. 1988) (Fig. 1b).

A change from aerobic to anoxic root metabolism may have serious consequences, particularly with respect to energy (ATP) production in the roots. Hypoxia does not appear to cause a root energy deficiency in higher plants, however, if alcoholic fermentation is stimulated and maintained at sufficiently high levels (Mendelssohn et al. 1981; Saglio et al. 1983; Mendelssohn and McKee 1987). The maintenance of a high AEC during hypoxia could occur through accelerated consumption of glucose (Pasteur effect) or by decreased ATP utilization (Mendelssohn and Burdick 1988). The maintenance of root energy status during hypoxia was most apparent in *S. alterniflora* (Fig. 1c). Although the total adenylate concentration and AEC ratio in *P. hemitomon* roots under hypoxia were lower than that in well-aerated roots, the differences were not significant (Fig. 1c).

The significant reduction in percent ^{15}N recovery in the hypoxic roots indicated that decreased aeration did affect nitrogen uptake by *S. alterniflora* and *P. hemitomon* (Fig. 1d). Soil waterlogging and hypoxia of culture solutions have been demonstrated to reduce nutrient uptake in nonwetland plant species (Drew and Sisworo 1977, 1979; Trought and Drew 1980). Previous work, however, has yielded conflicting results regarding the effect of hypoxia on nutrient uptake in *S. alterniflora*. Delaune et al. (1984) and Pezeshki et al. (1988) found no evidence of NH_4^+ uptake inhibition due to anaerobiosis in stirred sediment suspensions. Morris (1984) demonstrated a 60% decrease in root-specific uptake rates (V_{max}) of NH_4^+ by *S. alterniflora*, however, when exposed to anoxic conditions in hydroponic culture. Morris and Dacey (1984) further found that NH_4^+ uptake was reduced at low concentrations of root oxygen where aerobic respiration was inhibited.

A decreased energy status in the hypoxic roots cannot explain the inhibition of N uptake by *P. hemitomon* and *S. alterniflora* because both species maintained a relatively high AEC ratio in this treatment (Fig. 1c,d). Accelerated glycolysis, however, may result in a deficiency of carbon skeletons available for nitrogen metabolism. The end product of alcoholic fermentation, ethanol, apparently readily diffuses from the roots of *S. alterniflora* (Mendelssohn et al. 1981; Mendelssohn and McKee 1987), as well as other wetland macrophytes (Bertani et al. 1980), and would represent a major carbon drain from the plant, particularly if glucose consumption is substantially increased. Thus, even though stimulation of ADH activity appeared to aid in maintenance of root energy status in *S. alterniflora* and *P. hemitomon*, it may have resulted in a deficiency of carbon skeletons for NH_4^+ assimilation. The effect of hypoxia on NH_4^+ uptake is supported by the significantly lower leaf elongation rate relative to the plants in the aerated treatment (Fig. 1d). Further decreases in NH_4^+ uptake and leaf elongation occurred when sulfide was added to the hypoxic culture solution (Fig. 1d).

Nutrient uptake requires energy. Therefore, the decline of the root AEC ratios of *S. alterniflora* and *P. hemitomon* may have been an important factor in reducing root $^{15}NH_4^+$ uptake when sulfide was added to the culture media. This interpretation is supported by the fact that the same sulfide levels that suppressed AEC resulted in significantly lower N uptake in the roots (Fig. 1c,d). Percent recovery of ^{15}N was lower in the roots of both species after a brief exposure to sulfide treatments, whereas this response was seen in leaf tissue only in *P. hemitomon* (Table 2). The absence of an

effect of sulfide on *S. alterniflora* leaf ^{15}N recovery in this experiment may have been caused by the slower growth rate of this species relative to that of *P. hemitomon* (Fig. 1d). A longer term study may thus be needed to show significant differences in N transport to the leaf tissue as a result of sulfide toxicity in *S. alterniflora*.

Species-specific differences in pattern of response to sulfide indicated that the freshwater marsh species, *P. hemitomon*, was more sensitive to the presence of this phytotoxin than was the salt-marsh species, *S. alterniflora*. At sulfide concentrations of 1.0 mM and above, root energy status, NH_4^+ uptake, and leaf growth of *P. hemitomon* were more negatively affected relative to aerated controls. These results support earlier findings that showed similar differences in sulfide tolerance between these two species (Koch and Mendelssohn 1989). Work with other wetland macrophytes has also shown differential tolerance of sulfide and coincidence of sensitivity with species distribution in the field (Havill et al. 1985).

The precise mechanism whereby sulfide exerts an effect on plant growth is not well understood. A decrease in the activity of root metallo-enzymes has been postulated as the phytotoxic effect of H_2S (Allam and Hollis 1972; Havill et al. 1985; Pearson and Havill 1988) primarily because important oxidases such as cytochrome oxidase—the terminal enzyme in the electron transport chain of aerobic respiration—are inhibited by sulfide. Wetland macrophytes growing in anoxic, waterlogged soils, where H_2S can accumulate often exhibit anoxic root metabolism, however, which is believed to contribute to energy production (e.g. Moquot et al. 1981; Saglio et al. 1983, 1988). Therefore, the inhibitory effect of sulfide on aerobic respiration (i.e. cytochrome oxidase) alone may not be sufficient to explain the growth inhibition and lethal response, i.e. "die-back," found in natural and managed wetland systems where sulfide accumulates (Okajima and Takagi 1955; Mendelssohn and McKee 1988). The significant inhibition of ADH activity in *S. alterniflora* and *P. hemitomon* roots at sulfide concentrations of 2.0 and 1.0 mM, respectively (Fig. 1b), supports the idea that in addition to aerobic respiratory enzymes, H_2S may also affect alternate anoxic pathways through inhibition of a key enzyme, ADH. Mendelssohn et al. (1981) found depressed root ADH activity within the die-back zone of a *S. alterniflora* marsh where Eh indicated strongly reducing conditions, but higher enzyme activities in adjacent zones where the soil was less reduced; they concluded that ADH activity in the die-back zone was inhibited by a soil toxin such as H_2S.

Since sulfide addition caused a significant decrease in culture solution Eh ($P < 0.01$; Fig. 1a) and, hence, an increase in the oxygen demand (reducing power) of the rooting media, these species were simultaneously exposed to two potential stresses: sulfide toxicity and root oxygen deficiency. Attempts to separate the effects of redox potential and sulfide in culture solution by adding reducing agents have been unsuccessful (Ingold and Havill 1984). A decrease in Eh due to sulfide additions would be expected to cause stimulation in root ADH activity at least equal to that in hypoxic rooting media because oxygen would be limiting in all cases. Instead, ADH activity was significantly inhibited in both species at low redox conditions where sulfide concentrations were high (Fig. 1a,b). At low sulfide concentrations (0.5 mM), however, a stimulatory effect on ADH activity was observed in *P. hemitomon*. Pearson and Havill (1988) also showed an increase in ADH activity in several wetland and nonwetland macrophytes when 0.10 mM sulfide was added to a culture solution (+190 mV). It is possible that these low sulfide concentrations increased the oxygen demand of the culture solution, but were not high enough to inhibit ADH activity. Thus, although our results indicate the possibility of a sulfide effect at low concentrations through a further increase in oxygen demand (decrease in Eh) of the rooting media, they also support a toxic effect at higher concentrations, as evidenced by significant inhibition in the root ADH activity. Concomitant decreases in root energy status (Fig. 1c) suggest the possibility that as ADH activity was suppressed by H_2S the resultant decrease in anoxic root metabolism, as well as any limited aerobic respiration, caused root adenylate levels and energy charge to decline. It is apparent that although hypoxia

alone did not cause a significant decrease in the energy status of *P. hemitomon* and *S. alterniflora* roots, hypoxia plus sulfide (≥1.0 or 2.0 mM, respectively) did.

A decrease in ^{15}N recovery and leaf elongation with increasing sulfide concentration (Fig 1d) provides direct evidence of sulfide-induced inhibition of N uptake in a freshwater and a salt-marsh species. Whether the effect of sulfide on NH_4^+ uptake (Fig. 1d) was indeed mediated through an inhibition of alcoholic fermentation and ATP production is not conclusive and requires further investigation. Nonetheless, our data support the hypothesis that sulfide limits the potential for root anoxic energy production and contributes to the growing body of information that implicates sulfide as a major growth-limiting factor for wetland plant species. Soil sulfide concentrations may, therefore, be acting in concert with other factors (e.g. salinity) in exerting control of macrophyte species distribution in wetlands.

Conclusions

Sulfide toxicity has been implicated as a causative factor in the die-back of European and North American salt marshes (Goodman and Williams 1961; Ingold and Havill 1984; Mendelssohn and McKee 1988). Although H_2S is known to reduce growth in plants, we are only beginning to understand how this phytotoxin affects metabolic functions in marine, brackish, and freshwater wetland plant species. Under aerobic conditions in the root, sulfide can bind with metallo-enzymes (Allam and Hollis 1972; Havill et al. 1985; Pearson and Havill 1988), reducing the respiratory capacity of the roots by denaturing important oxidases and lowering the potential for aerobic root energy production. The data presented here show that H_2S can inhibit ADH activity, thereby limiting the functioning of an important alternate, anoxic pathway. As plants lose their ability to produce ATP and maintain the energy status of root cells, important energy-dependent metabolic functions (e.g. nutrient uptake) may be limited. Thus, wetland plants exposed to strongly reducing soil conditions where sulfide accumulates may exhibit reduced growth and even die-back because of the suppression of anoxic, as well as aerobic, energy production.

References

ALLAM, A. I., AND J. P. HOLLIS. 1972. Sulfide inhibition of oxidases in rice roots. Phytopathology **62:** 634–639.

ARMSTRONG, W. 1979. Aeration in higher plants. Adv. Bot. Res. **7:** 225–332.

ATKINSON, D. E. 1968. Energy charge of adenylate pool as a regulatory parameter: Interaction with feedback modifiers. Biochemistry **7:** 4030–4034.

BERTANI, A., I. BRAMBILLA, AND F. MENEGUS. 1980. Effect of anaerobiosis on rice seedlings: Growth, metabolic rate, and fate of fermentation products. J. Exp. Bot. **31:** 325–331.

BURESH, R. J., E. R. AUSTIN, AND E. T. CRASWELL. 1982. Analytical methods in ^{15}N research. Fert. Res. **3:** 37–62.

CARLSON, P. R., AND H. FORREST. 1982. Uptake of dissolved sulfide by *Spartina alterniflora*: Evidence from natural sulfur isotope ratios. Science **216:** 158–165.

CONNELL, W. E., AND W. H. PATRICK, JR. 1968. Sulfate reduction in soil: Effects of redox potential and pH. Science **159:** 86–87.

CRAWFORD, R. M. M. 1982. Physiological responses to flooding, p. 453–477. *In* Encyclopedia of plant physiology. V. 12B. Springer.

DELAUNE, R. D., C. J. SMITH, AND W. H. PATRICK, JR. 1983. Relationships of marsh elevation, redox potential, and sulfide to *Spartina alterniflora* productivity. Soil Sci. Soc. Am. J. **47:** 930–935.

———, ———, AND M. D. TOLLEY. 1984. The effect of sediment redox potential on nitrogen uptake, anaerobic root respiration and growth of *Spartina alterniflora* Loisel. Aquat. Bot. **18:** 223–230.

DREW, M. C., AND E. J. SISWORO. 1977. Early effects of flooding on nitrogen deficiency and leaf chlorosis in barley. New Phytol. **79:** 567–571.

———, AND ———. 1979. The development of waterlogging damage in young barley plants in relation to plant nutrient status and changes in soil properties. New Phytol. **82:** 301–314.

GAMBRELL, R. P., AND W. H. PATRICK, JR. 1978. Chemical and microbial properties of anaerobic soils and sediments, p. 375–423. *In* D. D. Hook and R. M. M. Crawford [eds.], Plant life in anaerobic environments. Ann. Arbor Sci.

GOODMAN, P. J., AND W. T. WILLIAMS. 1961. Investigations into "die-back." J. Ecol. **49:** 391–398.

HAUCK, R. D. 1982. Nitrogen-isotope-ratio analysis, p. 735–776. *In* A. J. Page and D. R. Kenney [eds.], Methods of soil analysis, part 2. Am. Soc. Agron.

HAVILL, D. C., A. INGOLD, AND J. PEARSON. 1985. Sulfide tolerance in coastal halophytes. Vegetatio **62:** 279–285.

HOWES, B. L., W. H. DACEY, AND S. G. WAKEMAN. 1985. Effects of sampling technique on measurements of porewater constituents in salt marsh sediments. Limnol. Oceanogr. **30:** 221–227.

———, R. W. HOWARTH, J. M. TEAL, AND I. VALIELA. 1981. Oxidation–reduction potentials in a salt

marsh: Spatial patterns and interactions with primary production. Limnol. Oceanogr. **26:** 350–360.

INGOLD, A., AND D. C. HAVILL. 1984. The influence of sulfide on the distribution of higher plants in salt marshes. J. Ecol. **72:** 1043–1054.

JOSHI, M. M., I. K. A. IBRAHIM, AND J. P. HOLLIS. 1975. Hydrogen sulfide: Effects on the physiology of rice plants and relation to straithead disease. Phytopathology **65:** 1165–1170.

KEELEY, J. E. 1979. Population differentiation along a flood frequency gradient—physiological adaptations to flooding in *Nyssa sylvatica*. Ecol. Monogr. **49:** 89–108.

KING, G. M., M. J. KLUG, R. G. WIEGERT, AND A. G. CHALMERS. 1982. Relation of soilwater movement and sulfide concentration to *Spartina alterniflora* production in a Georgia salt marsh. Science **218:** 61–63.

KOCH, M. S., AND I. A. MENDELSSOHN. 1989. Sulphide as a soil phytotoxin: Differential responses in two marsh species. J. Ecol. **77:** 565–578.

LINTHURST, R. A., AND E. D. SENECA. 1980. The effects of standing water and drainage potential on the *Spartina alterniflora*-substrate complex in a North Carolina salt marsh. Estuarine Coastal Mar. Sci. **11:** 41–52.

MENDELSSOHN, I. A. 1979*a*. Influence of nitrogen level, form, and application method on the growth response of *Spartina alterniflora* in North Carolina. Estuaries **2:** 106–111.

———. 1979*b*. Nitrogen metabolism in the height forms of *Spartina alterniflora* in North Carolina. Ecology **60:** 574–584.

———, AND D. M. BURDICK. 1988. The relationship of soil parameters and root metabolism to primary production in periodically inundated soils, p. 398–428. *In* D. D. Hook et al. [eds.], The ecology and management of wetlands. V. 1. Croom Helm.

———, AND K. L. MCKEE. 1981. Determination of adenine nucleotide levels and adenylate energy charge ratio in two *Spartina* species. Aquat. Bot. **11:** 37–55.

———, AND ———. 1987. Root metabolic response of *Spartina alterniflora* to hypoxia, p. 239–253. *In* Plant life in aquatic and amphibious habitats. Brit. Ecol. Soc. Spec. Symp. Spec. Publ. 5. Blackwell.

———, AND ———. 1988. *Spartina alterniflora* dieback in Louisiana: Time-course investigation of soil waterlogging effects. J. Ecol. **76:** 509–521.

———, ———, AND W. H. PATRICK, JR. 1981. Oxygen deficiency in *Spartina alterniflora* roots: Metabolic adaptation to anoxia. Science **214:** 439–441.

———, AND E. D. SENECA. 1980. The influence of soil drainage on the growth of salt marsh cordgrass *Spartina alterniflora* in North Carolina. Estuarine Coastal Mar. Sci. **11:** 27–40.

MOQUOT, B. C., C. PRAT, C. MOUCHES, AND A. PRADET. 1981. Effect of anoxia on energy charge and protein synthesis in rice embryo. Plant Physiol. **68:** 636–640.

MORRIS, J. T. 1980. The nitrogen uptake kinetics of *Spartina alterniflora* in culture. Ecology **61:** 1114–1121.

———. 1984. Effects of oxygen and salinity on ammonium uptake by *Spartina alterniflora* Loisel and *Spartina patens*. J. Exp. Mar. Biol. **78:** 87–98.

———, AND J. W. H. DACEY. 1984. Effects of O_2 on ammonium uptake and root respiration by *Spartina alterniflora*. Am. J. Bot. **71:** 979–985.

NESTLER, J. 1977. Interstitial salinity as a cause of ecophenic variation in *Spartina alterniflora*. Estuarine Coastal Mar. Sci. **16:** 103–109.

ODUM, E. P., AND M. E. FANNING. 1973. Comparison of the productivity of *Spartina alterniflora* and *Spartina cynosuroides* in Georgia coastal marshes. Ga. Acad. Sci. Bull. **31:** 1–12.

OKAJIMA, H., AND S. TAKAGI. 1955. Physiological behavior of hydrogen sulfide in the rice plant. Tohoku J. Agric. Res. (Pt. 2) **6:** 89–99.

PEARSON, J., AND D. C. HAVILL. 1988. The effect of hypoxia and sulfide on culture grown wetland and non-wetland plants. 2. Metabolic and physiological changes. J. Exp. Bot. **39:** 431–439.

PERATA, P., F. LOSCHIAVO, AND A. ALPI. 1988. Ethanol production and toxicity in suspension cultured carrot cells and embryos. Planta **173:** 322–329.

PEZESHKI, S. R., R. D. DELAUNE, AND C. W. LINDAU. 1988. Interaction among sediment anaerobiosis, nitrogen uptake and photosynthesis of *Spartina alterniflora*. Physiol. Plant. **74:** 561–565.

POSTGATE, J. 1959. Sulphate reduction by bacteria. Annu. Rev. Microbiol. **13:** 505–520.

SAGLIO, P. H., M. C. DREW, AND A. PRADET. 1988. Metabolic acclimation to anoxia induced by low (2–4 kPa partial pressure) oxygen pretreatment (hypoxia) in root tips of *Zea mays*. Plant Physiol. **86:** 61–66.

———, P. RAYMOND, AND A. PRADET. 1983. Transport and root respiration of maize seedlings. Plant Physiol. **72:** 1035–1039.

SAS INST. CORP. 1982. SAS user's guide: Statistics. Version 5 Ed.

SMITH, A. M., AND T. AP REES. 1979. Pathways of carbohydrate fermentation in the roots of marsh plants. Planta **146:** 327–334.

SULLIVAN, M. L., AND F. C. DAIBER. 1974. Response in production of cordgrass, *Spartina alterniflora*, to inorganic nitrogen and phosphorus fertilizer. Chesapeake Sci. **15:** 121–123.

TROUGHT, M. C., AND M. C. DREW. 1980. The development of waterlogging damage in wheat seedlings (*Triticum aestivum* L.). 2. Accumulation and redistribution of nutrients by the shoot. Plant Soil **56:** 187–199.

TURNER, F. T., AND W. H. PATRICK, JR. 1968. Chemical changes in waterlogged soils as a result of oxygen depletion. Trans. 9th Int. Cong. Soil Sci. **4:** 53–63.

VALIELA, I., AND J. M. TEAL. 1974. Nutrient limitation in salt marsh vegetation, p. 547–563. *In* R. J. Reimold and W. H. Queen [eds.], Ecology of halophytes. Academic.

Submitted: 9 January 1989
Accepted: 24 May 1989
Revised: 27 December 1989