Limnol. Oceanogr., 44(4), 1999, 1155–1159
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Oxidation of sulfide by *Spartina alterniflora* roots

Abstract—Root tips from the marsh grass *Spartina alterniflora,* collected from areas of high and low pore-water sulfide, exhibited a substantial capacity to catalyze sulfide oxidation, as determined by closed-chamber respirometry. A large proportion of this catalysis was apparently nonenzymatic and was higher in roots of plants from the high-sulfide versus the low-sulfide site. Activity exhibiting characteristics of enzymatic sulfide oxidation was significantly higher in plants from the low-sulfide site. Results from elemental analysis of root tissue were consistent with the theory that metals play a role in nonenzymatic catalysis. These results indicate that estuarine plants may detoxify environmental sulfide via sulfide oxidation.

Hydrogen sulfide is a common metabolic poison that is abundant in marine-reducing environments. Sulfide (H_2S, HS^-, and S^{2-}) blocks aerobic respiration by inhibition of mitochondrial cytochrome *c* oxidase (Nicholls 1975; Wilson and Erecinska 1978) and spontaneously oxidizes in the presence of dissolved oxygen, thereby reducing oxygen availability. Despite this, many aerobic marine organisms can survive chronic sulfide exposure. The mechanisms that enable these organisms to avoid sulfide toxicity are beginning to be characterized. Most of the work in this area has involved marine invertebrates, many of which are symbiotic with sulfur-oxidizing chemoautotrophic bacteria (reviewed in Somero et al. 1989; Childress and Fisher 1992). Although plants are found in many of the same environments as the invertebrates that exhibit mechanisms of sulfide detoxification (e.g., salt marshes, eel-grass beds, and mangrove swamps), no studies have investigated the sulfide-detoxification mechanisms of marine plants.

One plant that is subject to chronic sulfide exposure is *Spartina alterniflora,* a dominant salt marsh grass. This grass extends roots into reduced sediments that are rich in sulfide (Carlson and Forrest 1982; King et al. 1982). It is well established that sulfide can have deleterious effects (e.g., Howes et al. 1986; Pearson and Havill 1988; Pezeshki et al. 1993; Pezeshki and Delaune 1996). Environmental sulfide enters the root tissues (Carlson and Forrest 1982) and can inhibit metalloenzymes, including cytochrome *c* oxidase (Allam and Hollis 1972; Havill et al. 1985). Sulfide exposure correlates with stunted growth in the field (King et al. 1982) and results in reduced growth, alcohol dehydrogenase activity, adenylate charge, and nitrogen uptake in laboratory experiments (Koch and Mendelssohn 1989; Koch et al. 1990). Although sensitive to sulfide, *S. alterniflora* is more tolerant of sulfide than are other freshwater marsh species that do not encounter elevated sulfide (Koch and Mendelssohn 1989; Koch et al. 1990). Thus, it is apparent that while sulfide is an important environmental stressor of *S. alterniflora,* physiological mechanisms of sulfide tolerance may be exhibited.

S. alterniflora may detoxify sulfide in ways similar to those exhibited by marine invertebrates. An important feature of the lifestyle of invertebrates that tolerate sulfidic environments is that they bridge oxic and anoxic environments. For example, invertebrates at deep-sea hydrothermal vents inhabit areas where sulfide-laden water mixes with oxygenated water. Sediment-dwelling invertebrates aerate their environment by pumping oxygenated waters into their burrows. Another strategy, exhibited by some symbiotic clams, is to live partially in aerated seawater and to extend part of the body into sulfide-rich sediments or fissures. The presence of oxygen allows sulfide to be oxidized to less toxic species, such as thiosulfate, sulfite, sulfate, and elemental sulfur. Although sulfide is spontaneously oxidized by oxygen, invertebrates have a variety of means of catalyzing sulfide oxidation in order to gain greater protective benefit and, in some cases, in order to allow sulfide oxidation to be coupled to the production of cellular energy. Mechanisms that enhance the rate of sulfide detoxification but that do not result in energy gain are sulfide oxidases and catalysis by heavy metals (reviewed in Somero et al. 1989). Mechanisms that can result in energetic gain are sulfide oxidation by bacteria symbionts (Cavanaugh et al. 1981; Felbeck et al. 1981) or mitochondrial sulfide oxidation (e.g., Powell and Somero 1986; Lee et al. 1996; Völkel and Grieshaber 1997).

Like sulfide-tolerant marine invertebrates, *S. alterniflora* bridge oxic and anoxic environments. In *S. alterniflora* and other aquatic plants, a well-developed aerenchyma system facilitates the transport of oxygen from the atmosphere to the roots, where oxidation of sulfide potentially reduces its toxicity (Teal and Kanwisher 1966; Hwang and Morris 1991; Arenovski and Howes 1992; Armstrong et al. 1994; Howes and Teal 1994). Thus, sulfide oxidation may be a mechanism that allows *S. alterniflora* to tolerate sulfide. It is not known whether *S. alterniflora* actively facilitate oxidation. This plant-mediated oxidation could be catalyzed by sulfide oxidases, metals, mitochondria, or hitherto uncharacterized mechanisms. In the present study, we measured the potential for sulfide oxidation in roots of *S. alterniflora* and then determined whether this capacity is catalyzed by known mechanisms.

S. alterniflora used in the first series of experiments were collected at Airport Marsh on Dauphin Island, Alabama, adjacent to a tidal channel (16‰). Pore-water sulfide in sediments associated with *S. alterniflora* at this site was high, ranging from 1.3–8.1 mM total sulfide (Lee et al. 1996). Entire plants with or without associated sediment were transported in seawater to the laboratory for further investigation. Roots of *S. alterniflora* were subjected to several rinses in seawater to remove associated sediments. Several tips of roots (1–3 cm; 11–44 mg dry weight) were excised and given a final rinse with filtered artificial seawater (FASW; Tropic Marin, pH ≈8.0), until no sediment particles were evident under a dissecting microscope. These tips were then placed in a closed-chamber respirometer (Cyclobios Oxygraph 67097) containing 6 ml stirred (500 rpm) air-equilibrated FASW at 20°C. Chamber pH was likely in the range of 8

(approximate pH of FASW stock) to 7.6 (lowest value recorded in chamber medium at the end of experiments). Samples were exposed to stepwise increases from 0.05 to 5 mM sulfide (ΣH_2S; the sum of all species present) by injecting microliter aliquots of freshly made 30-mM sodium sulfide into the respirometer, using a Hamilton syringe. The reaction of sulfide and oxygen was measured by following the disappearance of oxygen. This ''sulfide-dependent oxidation'' correlates with the disappearance of sulfide, although the stoichiometry will differ depending on the end products of oxidation (e.g., $S_2O_3^{-2}$, SO_3^{-2}, and/or SO_4^{-2}). After experiments, root tips were blotted dry and then dried in a drying oven at 70°C for 24–48 h.

Root tips greatly accelerated sulfide-dependent oxidation in closed-chamber respirometry experiments. In chambers containing seawater only, low rates of background sulfide-dependent oxidation were observed (ranging from 20–300 pmol O_2 consumed per second) (Fig. 1A). Rates of background sulfide-dependent oxidation increased in a concentration-dependent manner, with higher rates observed in the presence of the highest sulfide concentrations tested, e.g., 5 mM. Oxygen-consumption rates in the presence of sulfide were considerably greater in chambers containing root tips (Fig. 1A). Without sulfide added, the rate of oxygen consumption due to aerobic respiration was 156 $\pm$ 54 pmol O_2 s^{-1} (SD; n = 13). Additions of sulfide resulted in increased oxygen consumption in a concentration-dependent manner (Fig. 1A). The increase over the rate at 0 mM sulfide (because of aerobic respiration) was as much as 1,400 pmol O_2 s^{-1} in the 5-mM sulfide treatment. Thus, the rate of oxygen consumption was accelerated up to 11-fold over rates of spontaneous oxygen consumption. These results demonstrate that *S. alterniflora* roots exhibit a substantial capacity for the catalysis of sulfide oxidation.

Root-tip sulfide-dependent oxidation was inhibited by 1-mM potassium cyanide (KCN), an electron-transport–chain inhibitor and inhibitor of chemical sulfide oxidation. In the presence of equimolar sulfide, KCN has been shown to reduce the rate of sulfide oxidation in water by >90% (Chen and Morris 1972). Following KCN preincubation for 8 h, the mass-specific rate of oxygen consumption by roots tips in the presence of sulfide and 1-mM KCN was considerably lower than that of non–KCN-treated tips (Fig. 1B). Rates of sulfide-dependent oxidation by KCN-treated roots were higher than background rates, which indicates that KCN did not completely inhibit sulfide oxidation. Although the exact mechanism of inhibition in these experiments is not known, it likely reflects inhibition of chemical and/or biological oxidation.

Further experiments were designed to assess the mechanism of sulfide oxidation in *S. alterniflora.* Sulfide oxidation may be catalyzed by mitochondria, bacteria associated with the root surface, sulfide-oxidase enzymes, and/or nonenzymatic catalysts such as metals. Boiling treatment should inactivate all but nonenzymatic catalysts. In order to measure the proportion of sulfide-dependent oxidation attributable to nonenzymatic catalysts, root tips from closed-chamber respirometry experiments were boiled for 10 min and were then reanalyzed. This treatment presumably destroyed mitochondrial, bacterial, and enzymatic activity but not nonenzyme catalysts. Following treatment by boiling, a substantial proportion of sulfide-dependent oxidation capacity remained, particularly at higher concentrations of sulfide (expressed as pmol O_2 consumed s^{-1} mg dry weight^{-1} and corrected for background sulfide-dependent oxidation rates; Fig. 2A,B). Thus, nonenzyme catalysts appear to contribute significantly to sulfide oxidation.

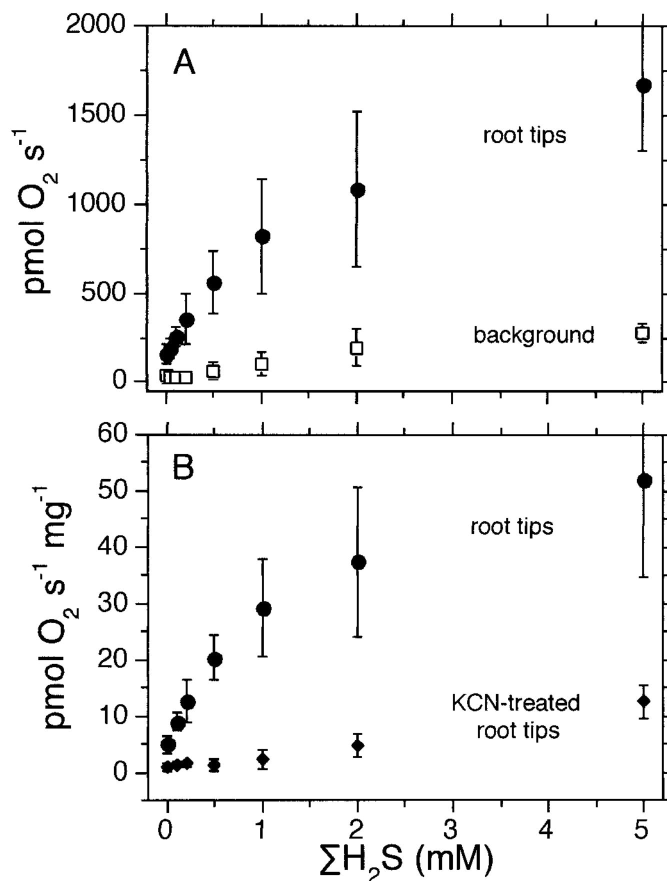

Fig. 1. Sulfide-dependent oxidation measured as oxygen consumption by closed-chamber respirometry. (A) Oxidation by root tips versus background oxidation: open circles = chamber containing filtered seawater only (n = 12 determinations); black circles = root tips (12–34 mg dry weight; n = 13 determinations from different root-tip samples) from *Spartina alterniflora* collected at Airport Marsh, Dauphin Island, Alabama. Root-tip data are not expressed per milligram of dry weight in order to facilitate comparison with background rates. (B) Effect of KCN (sulfide oxidation inhibitor): black circles = root tips; black diamonds = 1-mM KCN–incubated root tips (24–44 mg) from *S. alterniflora* collected at Airport Marsh (n = 3 determinations from different root-tip samples). Rates of sulfide-dependent oxidation were measured as oxygen consumption per milligram of dry weight and were corrected for rates of background sulfide-dependent oxidation exhibited by seawater-only controls. Data points represent means $\pm$ 1 SD.

The proportion of sulfide-dependent oxidation that was sensitive to thermal denaturation ranged from 80% at 0.05-mM sulfide to 0% at 5-mM sulfide. This heat-labile sulfide-dependent oxidation (Fig. 2C) may be the result of mitochondria, bacteria, and/or sulfide oxidases. Whether this heat-labile activity reflects higher rates of sulfide disappear-

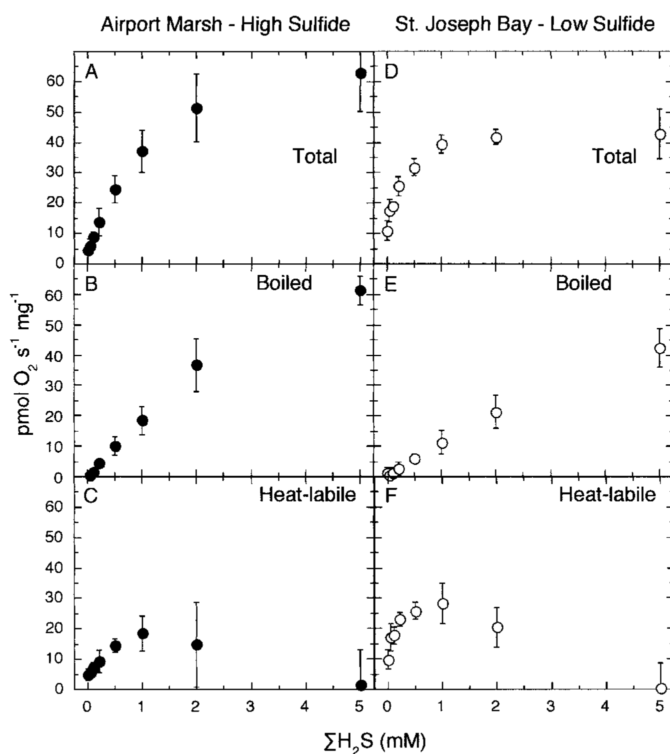

Fig. 2. Sulfide-dependent oxidation by root tips of *Spartina alterniflora* collected at Airport Marsh (black circles; high-sulfide environment) and St. Joseph Bay (open circles: low-sulfide environment). Rates of sulfide-dependent oxidation were measured as oxygen consumption per milligram of dry weight and were corrected for rates of background sulfide-dependent oxidation exhibited by seawater-only controls. (A, D) Sulfide-dependent oxidation by untreated roots or total oxidation by all catalysts present in root tips. (B, E) Sulfide-dependent oxidation following boiling treatment (10 min) to inactive mitochondrial, bacterial, and/or enzymatic catalysis. (C, F) Heat-labile catalysis (total − boiled). Data points are means ± 1 SD of three to four determinations from different root-tip samples.

ance or production of more oxidized end products could not be determined from our analyses. Heat-labile sulfide-dependent oxidation accounted for the majority of sulfide-dependent oxidation at lower sulfide concentrations, with a maximum rate in the presence of 1-mM sulfide. Heat-labile sulfide-dependent oxidation was inhibited at sulfide concentrations of greater than 1 mM. Thus, at least two types of sulfide-detoxification mechanisms may operate in *S. alterniflora* roots: heat-labile catalysis, which could be coupled to energetic gain, at low-to-moderate sulfide concentrations and nonenzymatic catalysis at higher sulfide concentrations.

In order to test whether sulfide oxidation capacity is correlated with environmental sulfide concentrations, parallel experiments were conducted on root tips from *S. alterniflora,* collected from sandy sediments in St. Joseph Bay, Florida. Sediment pore water at this collection site contains around 30 μM sulfide, on average (Powell, pers. comm.). Plants were collected and closed-chamber respirometry of root tips was conducted, as described above. Root tips from St. Joseph Bay plants were also able to catalyze sulfide-dependent oxidation, but the kinetics differed from those of the Airport Marsh roots. Sulfide-dependent oxidation rates at sulfide concentrations below 1 mM were higher than those observed in roots from Airport Marsh (Fig. 2A,D). Maximal rates at 2 and 5 mM sulfide of St. Joseph Bay roots were lower (Fig. 2A,D). Boiling-insensitive catalysis of roots from St. Joseph Bay was significantly lower than in Airport Marsh roots at 0.05-, 2-, and 5-mM sulfide concentrations (analysis of variance [ANOVA], $P < 0.05$; Fig. 2B,E). In contrast, rates of heat-labile catalysis of St. Joseph Bay roots were significantly higher than in Airport Marsh roots for 0.05–0.5-mM sulfide treatments (ANOVA, $P < 0.05$; Fig. 2C,F). Differences in sulfide-dependent catalysis between sites reflect differences in sulfide-consumption rates and/or oxidation states of the end products. The characteristics of sulfide-dependent oxidation in root tips collected from St. Joseph Bay reflect exposure to lower sulfide levels. Heat-labile sulfide-dependent oxidation, which appears to function best at low-to-moderate sulfide concentrations, was enhanced, while nonenzymatic sulfide-dependent oxidation, which may be more important at higher sulfide concentrations, was diminished.

The specific enzymes or nonenzyme catalysts of sulfide oxidation in sulfide-tolerant organisms have not been characterized. Sulfide oxidation is completely inactivated by thermal denaturation in some (e.g., *Geukensia demissa* [Lee et al. 1996]) but not all sulfide-tolerant invertebrates. In other species, up to 30–50% of total activity remains after boiling (Powell and Somero 1985; Martineu and Juniper 1997) and may be catalyzed by metals. A similar mechanism may also be present in *S. alterniflora.* A correlation between metal levels and rates of nonenzymatic sulfide oxidation has not been previously documented; this process is complicated by the fact that methods commonly employed to quantify levels of metals in organisms do not resolve the inorganic species involved or whether the metals are complexed to organic molecules. As a first step in assessing the potential role of metals in catalyzing sulfide oxidation, further analyses of *S. alterniflora* roots were conducted in order to determine whether metal catalysts are present.

Scanning electron micrographs of an Airport Marsh *S. alterniflora* root sample did not show dense particle accumulation or root coatings (data not shown), as observed in some *S. alterniflora* by Mendelssohn and Postek (1982). Energy-dispersive X-ray (EDX) analysis of the few particles that were evident did not reveal detectable levels of iron (Fe), although a large calcium (Ca) peak was observed in a scan of one particle and a large aluminum (Al) peak in the scan of another. Fe was detected in scans of epidermis and cortex and appeared similar between these tissue types. Because metal concentrations associated with the root tips used in our study were generally below detection limits, the localization of metals could not be determined by EDX.

Total metal concentrations were measured in *S. alterniflora* root tips, and comparisons were made between samples from Airport Marsh and St. Joseph Bay, since differences were observed between sites in terms of nonenzymatic catalysis. Dried root samples used in our respirometry experiments were analyzed by inductively coupled argon plasma spectrometry (ICAP, Jarrell-Ash 967 Atomcomp). Results from 20-element ICAP scans performed on dry-ashed samples pooled from three to four respirometric runs are given

Table 1. Element concentrations of *Spartina alterniflora* root tips from respirometry experiments determined by inductively coupled argon plasma emission spectrometry. Values for apple leaves, which were used as a standard in these analyses, are included for comparison with *S. alterniflora.* All values are in μg g dry weight^{-1} and presented as mean + SD where applicable. Boldface denotes elements in which significant differences were observed between collection sites (ANOVA; $P < 0.05$).

	Site		
Element	Airport Marsh ($n = 5$)	St. Joseph Bay ($n = 3$)	Apple leaves ($n = 2$)
Al*	**1,378 ± 282**	**166 ± 34**	288
As	9 ± 8	1 ± 2	5
B	25 ± 4	34 ± 9	29
Ba	5 ± 1	4 ± 1	48
Ca	1,465 ± 274	1,752 ± 468	15,148
Cd	1 ± 0.5	1 ± 0.3	0.4
Co	5 ± 5	1 ± 0.3	0.6
Cr	11 ± 3	11 ± 5	4
Cu	10 ± 3	6 ± 3	6
Fe*	**857 ± 332**	**149 ± 24**	77
K	1,039 ± 9,937	16,458 ± 13,336	16,517
Mg*	**3,077 ± 715**	**4,283 ± 106**	1,892
Mn*	**50 ± 30**	**9 ± 4**	56
Na*	**13,764 ± 2,509**	**25,073 ± 3,325**	64
Ni	10 ± 8	6 ± 1	3
P	823 ± 245	798 ± 445	1,776
Pb*	**9 ± 2**	**5 ± 1**	4
Si*	**1,736 ± 343**	**319 ± 89**	451
Sr	28 ± 5	33 ± 10	25
Zn*	**38 ± 20**	**7 ± 1**	11

* Denotes elements for which significant differences were observed between the Airport Marsh and St. Joseph Bay collection sites. (ANOVA; $P < 0.05$).

in Table 1. Ca, potassium, magnesium, and sodium were the most abundant of the elements analyzed. Al and Fe were the most abundant trace metals. Al, copper (Cu), Fe, manganese (Mn), and zinc (Zn) of roots from Airport Marsh *S. alterniflora* were similar in value to those same metals in *S. alterniflora* from Georgia salt marshes (Alberts et al. 1990). Trace-metal concentrations were generally lower in St. Joseph Bay *S. alterniflora,* with significantly lower concentrations of Al, Fe, Mn, lead, and Zn (ANOVA; $P < 0.05$).

Oxides of Fe^{2+}, Fe^{3+}, Fe, Mn^{+2}, and Mn as well as Cu^{+2}, cadmium $(Cd)^{2+}$, nickel $(Ni)^{+2}$, cobalt $(Co)^{2+}$, and Ca^{+2} are known to accelerate sulfide oxidation (Chen and Morris 1972; Millero 1991; Zhang and Millero 1993). If metals play a role in sulfide oxidation by *S. alterniflora* roots, Fe and Mn appear to be likely catalysts, since they are abundant, likely present in catalytically active form, and are higher in plants from Airport Marsh compared with plants from St. Joseph Bay. Involvement of Cu^{+2}, Cd^{2+}, Ni^{+2}, Co^{2+}, and Ca^{+2} cannot be ruled out. Thus, our results give correlative evidence that metals are involved in sulfide oxidation.

Whether the capacity of *S. alterniflora* to catalyze sulfide oxidation serves a protective function or exacerbates the problem of low oxygen availability is dependent upon the redox conditions of the rhizosphere. If gas transport via the aerenchyma system exceeds aerobic demands, then some oxygen may be available to oxidize sulfide and to diffuse into the surrounding sediment. Although oxygen loss from the rhizosphere was not observed in laboratory experiments (Howes and Teal 1994), geochemical evidence indicates that root-oxygen loss clearly occurs (Howes and Teal 1994). However, *S. alterniflora* roots also have a well-developed capacity for anaerobic metabolism, indicating that periodic or even chronic anoxia can be encountered, particularly in highly reduced sediments (Mendelssohn et al. 1981). Recent quantitative 16S ribosomal ribonucleic acid–probing studies indicate that sulfate-reducing bacteria are an important component of the microbial community associated with root surfaces of *S. alterniflora* (Rooney-Varga et al. 1997) and that they are, again, suggestive of periodic or chronic conditions of anoxia. Since the redox conditions of the rhizosphere appear to be quite dynamic, the role of sulfide-oxidation mechanisms in *S. alterniflora* roots will change accordingly. Under anoxic conditions, mechanisms of sulfide oxidation observed in this study, in which sulfide is oxidized by dissolved oxygen, are likely nonfunctioning. Under conditions in which aerobic respiration is limited by oxygen supply, the catalysis of sulfide likely has the deleterious effect of further reducing oxygen levels. Under conditions in which oxygen transport exceeds aerobic respiration, sulfide oxidation likely protects aerobic respiration.

Mechanisms of sulfide oxidation are probably widespread in aquatic plants that encounter sulfide and are likely to be of ecological significance. We need to learn more about these mechanisms, especially from the standpoint of understanding how dissimilar organisms, such as marsh plants and aquatic invertebrates, adapt to similar environmental challenges. A significant proportion of sulfide oxidation in *S. alterniflora* roots is heat labile and may be catalyzed by mitochondria, associated bacteria, and/or sulfide-oxidase enzymes. Of particular interest is the possibility of mitochondrial sulfide oxidation. The coupling of sulfide oxidation to oxidative phosphorylation of adenosine 5′-diphosphate has been demonstrated in the mitochondria isolated from a variety of animals that encounter sulfide (Powell and Somero 1986; Bagarinao and Vetter 1990; Oeschger and Vismann 1994; Völkel and Grieshaber 1997) and may occur in mitochondria in situ (Lee et al. 1996). If a similar mechanism is present in *S. alterniflora,* sulfide, which has previously only been recognized as a phytotoxin, may provide a benefit (under some conditions) by serving as a source of usable cellular energy.

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Acknowledgments

Technical assistance and instrumentation for SEM-EDX analyses provided by R. D. Griffin and M. C. Koopman at the Department of Materials Science and Engineering, University of Alabama at Birmingham. ICAP analyses were conducted by L. Berger–Bishop at the Chemical Analysis Laboratory, University of Georgia. This work was supported in part by NSF grant IBN9219658 to J.E.D. and D.W.K.

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References

ALBERTS, J. J., M. T. PRICE, AND M. KANIA. 1990. Metal concentrations in tissues of *Spartina alterniflora* (Loisel.) and sediments of Georgia salt marshes. Estuar. Coast. Shelf Sci. **30:** 47–58.

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

ARENOVSKI, A. L., AND B. L. HOWES. 1992. Lacunal allocation and gas transport capacity in the salt marsh grass *Spartina alterniflora.* Oecologia **90:** 316–322.

ARMSTRONG, W., R. BRANDLE, AND M. B. JACKSON. 1994. Mechanisms of flood tolerance in plants. Acta Bot. Neerl. **43:** 307–358.

BAGARINAO, T., AND R. D. VETTER. 1990. Oxidative detoxification of sulfide by mitochondria of the California killifish *Fundulus parvipinnis* and the speckled sanddab *Citharichthys stigmaeus.* J. Comp. Physiol. B **160:** 519–527.

CARLSON, P. R., AND J. FORREST. 1982. Uptake of dissolved sulfide by *Spartina alterniflora*: Evidence from natural sulfur isotope abundance ratios. Science **216:** 633–635.

CAVANAUGH, C. M., S. L. GARDINER, M. L. JONES, H. W. JANNASCH, AND J. B. WATERBURY. 1981. Prokaryotic cells in the hydrothermal vent tube worm *Riftia pachyptila*: Possible chemoautotrophic symbionts. Science **213:** 340–342.

CHEN, K. Y., AND J. C. MORRIS. 1972. Oxidation of sulfide by O_2: Catalysis and inhibition. J. Sanit. Eng. Div. Am. Soc. Civil Eng. **98 (SA1):** 215–227.

CHILDRESS, J. J., AND C. R. FISHER. 1992. The biology of hydrothermal vent animals: Physiology, biochemistry and autotrophic symbioses. Oceanogr. Mar. Biol. Annu. Rev. **30:** 337–441.

FELBECK, H., G. N. SOMERO, AND J. J. CHILDRESS. 1981. Calvin–Benson cycle sulphide oxidation enzymes in animals from sulphide rich habitats. Nature **293:** 291–293.

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

HOWES, B. L., J. W. H. DACEY, AND D. D. GOEHRINGER. 1986. Factors controlling the growth form of *Spartina alterniflora*: Feedbacks between above-ground production, sediment oxidation, nitrogen and salinity. J. Ecol. **74:** 881–898.

———, AND J. M. TEAL. 1994. Oxygen loss from *Spartina alterniflora* and its relationship to salt marsh oxygen balance. Oecologia **97:** 431–438.

HWANG, Y., AND J. T. MORRIS. 1991. Evidence for hydrometric pressurization in the internal gas space of *Spartina alterniflora.* Plant Physiol. **96:** 166–177.

KING, G. M., M. J. KLUG, R. G. WIEGERT, AND A. G. CHALMERS. 1982. Relation of soil water 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.

———, ———, AND K. L. MCKEE. 1990. Mechanism for the hydrogen sulfide-induced growth limitation in wetland macrophytes. Limnol. Oceanogr. **35:** 399–408.

LEE, R. W., D. W. KRAUS, AND J. E. DOELLER. 1996. Sulfide-stimulation of oxygen consumption rate and cytochrome reduction in gills of the estuarine mussel *Geukensia demissa.* Biol. Bull. **191:** 421–430.

MARTINEU, P., AND S. K. JUNIPER. 1997. Comparison of the benzyl viologen and bimane HPLC assays for the determination of sulfide-oxidizing capability in the tissues of hydrothermal vent and non-vent polychaetes. Can. J. Zool. **75:** 1618–1627.

MENDELSSOHN, I. A., K. L. MCKEE, AND W. H. PATRICK, JR. 1981. Oxygen deficiency in *Spartina alterniflora* roots: Metabolic adaptation to anoxia. Science **214:** 439–441.

———, AND M. T. POSTEK. 1982. Elemental analysis of deposits on the roots of *Spartina alterniflora* Loisel. Am. J. Bot. **69:** 904–912.

MILLERO, F. J. 1991. The oxidation of H_2S in Black Sea waters. Deep-Sea Res. **38:** S1139–S1150.

NICHOLLS, D. G. 1975. The effect of sulphide on cytochrome aa_3. isoteric and allosteric shifts of the reduced alpha-peak. Biochim. Biophys. Acta **396:** 24–35.

OESCHGER, R., AND B. VISMANN. 1994. Sulphide tolerance in *Heteromastus filiformis* (Polychaeta): Mitochondrial adaptations. Ophelia **40:** 147–158.

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

PEZESHKI, S. R., AND R. D. DELAUNE. 1996. Responses of *Spartina alterniflora* and *Spartina patens* to rhizosphere oxygen deficiency. Acta Oecol. **17:** 365–378.

———, J. H. PARDUE, AND R. D. DELAUNE. 1993. The influence of soil oxygen deficiency on alcohol dehydrogenase activity, root porosity, ethylene production and photosynthesis in *Spartina patens.* Environ. Exp. Bot. **33:** 565–573.

POWELL, M. A., AND G. N. SOMERO. 1985. Sulfide oxidation occurs in the animal tissue of the gutless clam, *Solemya reidi.* Biol. Bull. **169:** 164–181.

———, AND ———. 1986. Hydrogen sulfide oxidation is coupled to oxidative phosphorylation in mitochondria of *Solemya reidi.* Science **233:** 563–566.

ROONEY-VARGA, J. N., R. DEVEREUX, R. S. EVANS, AND M. E. HINES. 1997. Seasonal changes in the relative abundance of uncultivated sulfate-reducing bacteria in a salt marsh sediment and in the rhizosphere of *Spartina alterniflora.* Appl. Environ. Microbiol. **63:** 3895–3901.

SOMERO, G. N., J. J. CHILDRESS, AND A. E. ANDERSON. 1989. Transport, metabolism, and detoxification of hydrogen sulfide in animals from sulfide-rich marine environments. Rev. Aquat. Sci. **1:** 591–614.

TEAL, J. M., AND J. W. KANWISHER. 1966. Gas transport in the marsh grass, *Spartina alterniflora.* J. Exp. Bot. **17:** 355–361.

VÖLKEL, S., AND M. K. GRIESHABER. 1997. Sulphide oxidation and oxidative phosphorylation in the mitochondria of the lugworm *Arenicola marina.* J. Exp. Biol. **200:** 83–92.

WILSON, D. F., AND M. ERECINSKA. 1978. Ligands of cytochrome *c* oxidase. Methods Enzymol. **53:** 191–201.

ZHANG, J., AND F. J. MILLERO. 1993. The products from the oxidation of H_2S in seawater. Geochim. Cosmochim. Acta **57:** 1705–1718.

Received: 23 May 1997
Accepted: 23 September 1998
Amended: 26 February 1999