SULPHIDE UTILIZATION AND INJURIES IN HYPOXIC ROOTS AND RHIZOMES OF COMMON REED (*PHRAGMITES AUSTRALIS*)

Konrad Fürtig, Adrian Rüegsegger, Christian Brunold & Roland Brändle

Institute of Plant Physiology, University of Bern, Altenbergrain 21, 3013 Bern, Switzerland; tel. +41 31 631 49 56, fax +41 31 332 20 59, E-mail RBRAENDLE@PFP.UNIBE.CH

Abstract: The presented investigations have been carried out in order to estimate toxic sulphide levels and to examine detoxification capabilities in roots and rhizomes of the common reed (*Phragmites australis*).

Underground organs of common reed are sensitive towards sulphide above 1 mM applied exogenously under hypoxia. However, certain tolerance may be achieved by sulphide detoxification. Accumulated sulphide is partially used for the synthesis of non-toxic thiols, mainly glutathione. But the detoxification capacity of the underground organs is limited. Maximum concentrations of thiols are about 60 nmol/g^{-1} fw in roots and 300 nmol/g^{-1} fw in rhizomes.

Energy metabolism is considerably affected by low sulphide concentrations of 1 mM for 4 days, and immediately disturbed by increased concentrations up to 6 mM sulphide. Adenylate energy charge, total adenylates, posthypoxic respiration, and fermentation capacity decrease significantly. Roots are more sensitive than rhizomes.

INTRODUCTION

Flooded sediments are usually oxygen free. Reduced sediments of eutrophic lakes often accumulate phytotoxic compounds such as sulphide. It is generated by microbial sulphate respiration and by incomplete decomposition of organic matter (MELZER & STEINBERG 1983). Sulphide is supposed to inactivate metalloenzymes by the formation of the corresponding metal sulphides (HOCK & ELSTNER 1988), or to cleave intramolecular disulphide bonds of proteins (VISMANN 1991). However, sulphide rich environments have been shown to interfere with metabolism, ion uptake, and growth of *Spartina alterniflora* LOISEL. and other saltmarsh plants (PEARSON & HAVILL 1988, PEZESHKI et al. 1988, KOCH & MENDELSSOHN 1989, BRADLEY & MORRIS 1990, KOCH et al. 1990). Hence, it might be possible, in eutrophic stands of *Phragmites australis* (CAV.) TRIN. ex STEUD., that sulphide also impairs the metabolism of reed underground organs and therefore contributes to the reed decline that is observed in numerous European lakes (OSTENDORP 1989). However, the information on sulphide action alone is rather scarce. Experiments with controlled sulphide application could provide further information to characterize the detoxification capabilities, and to gain an insight into the sulphide effects on aspects of energy metabolism.
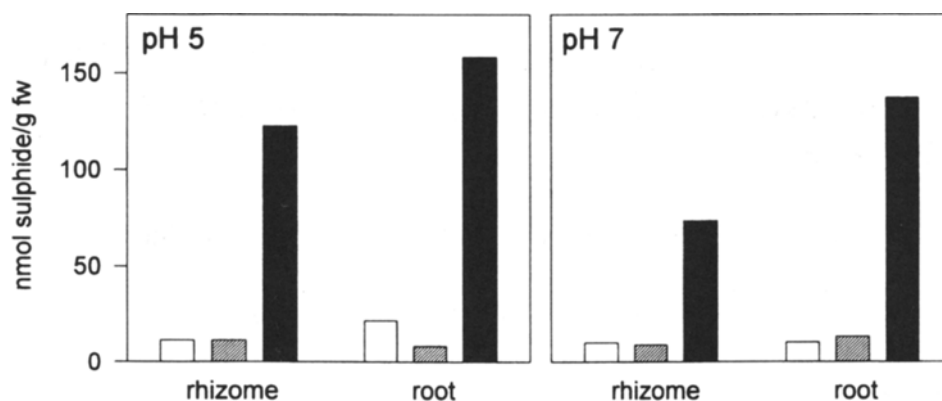
Fig. 1. Sulphide content of reed (*Phragmites australis*) roots and rhizomes after 24 h incubation at pH 5 or 7. Mean of 2 independent experiments. ☐ – air saturated nutrient solution, ▨ – hypoxia (< 0.6 mg O₂/l), ■ – hypoxia 1 mM Na₂S.

MATERIAL AND METHODS

Potted plants of common reed (*Phragmites australis* from Bestmann Ing., Wedel/Holstein, Germany) were pre-cultivated at 20 °C under 14 h light $(44 \cdot 10^{18} \text{ Q/m}^{-2}\text{s}^{-1})$ and 10 h dark regime. The root/rhizome systems, filling in volumes of about 8 × 8 × 10 cm, were kept submersed in slightly fertilized tap water. Before incubation the roots and rhizomes of plants with 2 to 4 shoots (length 30-70 cm) were carefully rinsed with tap water and pre-incubated for 2 h in either aerated or de-oxygenated water.

The experiments took place under the same conditions in slightly buffered nutrient solutions containing 3 mM K/Na phosphate buffer, pH 5 or 7, 0.75 mM MgSO₄ and 1.5 mM Ca(NO₃)₂. For some experiments 2 mM 2-(N-morpholino)ethanesulfonic acid (= MES) was added to support the buffer capacity. The root/rhizome bulk was incubated in a tightly closed flow-through system in order to keep oxygen concentrations low, and to maintain sulphide and pH values stable during the hypoxic treatment. The nutrient solution percolated with a flow rate that exchanged the incubation medium threefold per day. Liquid loss from the reservoir was replenished by nitrogen thus minimizing oxygen influx into the system. The medium of the normoxic controls had access to air. The media around the roots and rhizomes were permanently stirred. The shoots were exposed to air and light. The oxygen concentration could be kept below 0.6 mg O₂/l, and only very little sulphide was lost during incubation. Sulphide was added as gently washed Na₂S crystals. Oxygen and sulphide concentrations and pH values of the medium were monitored at the beginning and at the end of the treatment. Sulphide was determined according to SIEGEL (1965). Oxygen was measured with electrodes. The following sulphide insensitive oxygen sensor was kindly made available by J. Pokorný, Třeboň, Czech Republic: Gas voltametric sensor with separated reference electrode, author certificate CS.A.O. 01118/87 No. 267 864, Čáp J., Pokorný J., Šerák L., Teffer V. After incubation the total fractions of rhizomes and roots were used either fresh or deep frozen in liquid nitrogen and pulverized in a dismembrator (Mikro II, Braun, Melsungen, Germany).
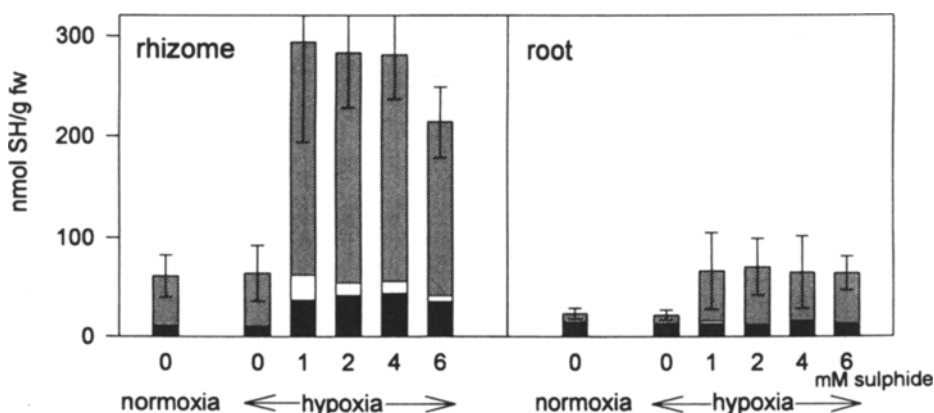
Fig. 2. Thiol contents after 24 h incubation under normoxia or hypoxia with increasing sulphide concentrations at pH 7. Mean of 5 independent experiments ± standard deviation of the sum. ■ – cysteine, ☐ – γ-EC,▨ – GSH.

Sulphide of the plant material was also determined according to SIEGEL (1965). Before freezing and pulverization, attached sulphide was removed by cold 0.1 M HCl and cold distilled water. The frozen powder was suspended in de-oxygenated 0.1 M NaOH. The extract was used directly for the staining reaction. The amount of reduced thiols was determined according to GRILL & ESTERBAUER (1973). Endogenous sulphide that interferes with the test was removed from the extract by bubbling nitrogen. The individual thiols (glutathione = GSH, γ-glutamylcysteine = γ-EC, and cysteine) were assayed according to RÜEGSEGGER & BRUNOLD (1992). GSH was used as reference. Adenylate energy charge (= AEC) and total adenylates were measured as described by SIEBER & BRÄNDLE (1991). The post-hypoxic respiration capacity of tissue slices was determined with the help of a respirometer (Gilson, Villiers-le-Bel, France). The extractable alcohol dehydrogenase (= ADH) activity was assayed according to BRÄNDLE (1983), starting with 0.1 M ethanol instead of 1.4 M. Most values are given on a fresh weight base, except for respiration and ADH measurements where proteins were used as references (BRADFORD 1976).

RESULTS

Sulphide uptake and detoxification

Sulphide is taken up out of stirred hypoxic solutions in comparable amounts at pH 5 (H_2S 90% of total sulphide) and pH 7 (H_2S 50% and HS^- 50%). It can be detected in roots as well as rhizomes (Fig. 1). The application of 1 mM sulphide for 24 h causes mean concentrations of 140-165 nmol/g^{-1} fw in roots, and 80-130 nmol/g fw in rhizomes. That is equivalent to about 0.15-0.25 mM of the tissue water content, provided that sulphide allocation is uniform. The higher pH value was chosen for further investigations because it represents the situation in most lakes of the central part of Switzerland.

However, sulphide treatment increases the content of non-protein thiols as well in roots and rhizomes, indicating the detoxification of sulphide (Fig. 2). Oxygen deficit stress per se
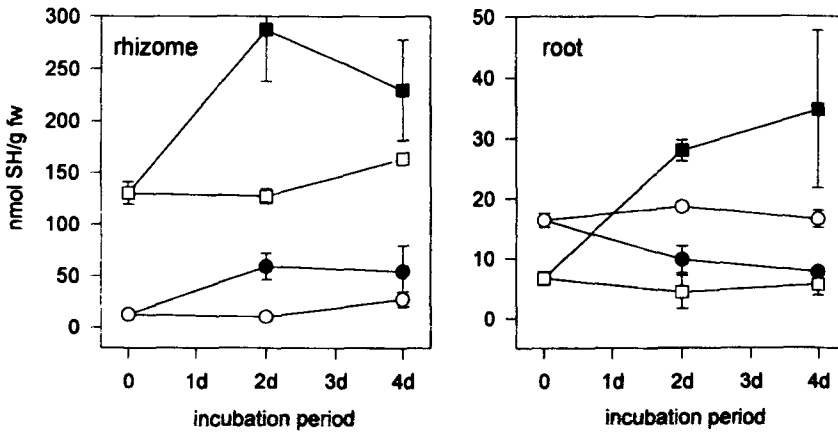
Fig. 3. Time dependent thiol accumulation in hypoxic roots and rhizomes fed without sulphide (O–O – cysteine, □–□ – GSH) or with 1 mM sulphide (●–● – cysteine, ■–■ – GSH), d – days. Mean of 3 independent experiments ± standard deviation.
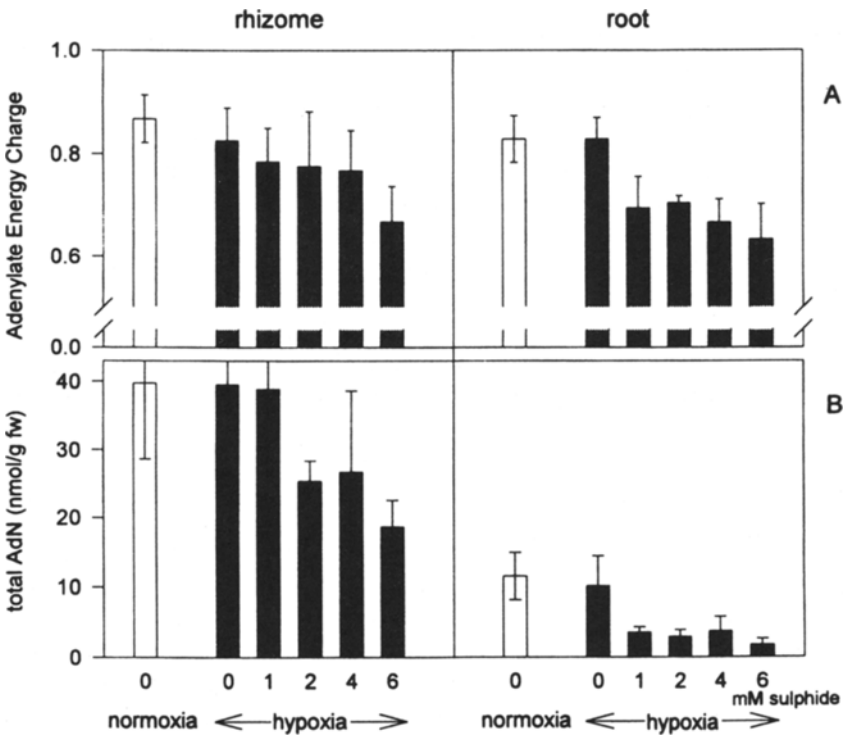

Fig. 4. Adenylate energy charge (A) and concentration (B) of total adenylates after 24 h normoxia or hypoxia with increasing sulphide concentrations. Mean of 5 independent experiments ± standard deviation.

Table 1. Time dependent decrease of adenylate energy charge and total adenylates (ATP+ADP+AMP) in roots and rhizomes treated with 1 mM sulphide under hypoxia (< 0.6 mg O_2/l). The values of the adenylate content are given as percentage in relation to the corresponding sulphide free treated control plants (fw as reference). Mean of 3 independent experiments ± standard deviation.

| | Adenylate energy charge | | | Total adenylate (%) |
| | hypoxia | | hypoxia + 1 mM Na_2S | hypoxia + 1 mM Na_2S |
Incubation time				
Rhizome				
0		0.90 (± 0.05)		100
1 day	0.82 (± 0.07)		0.78 (± 0.08)	116 (± 35)
2 days	0.82 (± 0.09)		0.76 (± 0.04)	106 (± 13)
4 days	0.81 (± 0.07)		0.71 (± 0.13)	77 (± 43)
Root				
0		0.93 (± 0.03)		100
1 day	0.88 (± 0.07)		0.68 (± 0.04)	71 (± 47)
2 days	0.89 (± 0.07)		0.84 (± 0.06)	56 (± 16)
4 days	0.87 (± 0.05)		0.79 (± 0.10)	18 (± 7)

has no influence. Rhizomes contain more thiols than roots. Concentrations of 1 mM sulphide give rise to a five-fold increase of thiol content in rhizomes (200-300 nmol SH/g fw) and a three- or four-fold increase in roots (50-60 nmol SH/g fw). Higher sulphide concentrations up to 6 mM do not exceed the maximum thiol concentrations reached before. The extension of the incubation period up to 4 days at 1 mM sulphide leads to the same maximum thiol concentration in rhizomes as already reached within 2 days. This increase is somewhat less pronounced in roots (Fig. 3). Obviously, the detoxification capacity is limited, and the efficiency of sulphide detoxification appears to be restricted to short-term exposure or to low concentrations. GSH, cysteine, and γ-EC represent virtually the total of all thiols detectable in reed rhizomes and roots as suggested by comparison of total amount of thiols (data not shown) and the sum of the individual thiols by different assays. The main fractions consist of glutathione (GSH) and cysteine.

Sulphide effects on energy metabolism

The adenylate energy charge (AEC = ([ATP]+1/2[ADP])/([ATP]+[ADP]+[AMP])) is a useful indicator to demonstrate the relationship between ATP regenerating and ATP utilizing processes, and therefore changes in the potential metabolic activity (PRADET & RAYMOND 1983). The values range from 0 to 1, and low values indicate generally a loss of viability. Sulphide exposure of common reed indeed causes an AEC decrease in roots and rhizomes (Fig. 4A). But even the treatment with concentrations up to 6 mM for 24 h cannot shift the AEC value below 0.6, and rather high AEC levels are also maintained during the long-term treatment with 1mM sulphide (Tab. 1). Nevertheless, these results indicate a reduced ATP availability. Furthermore, the content of adenylates declines continually with increasing sulphide concentrations (Fig. 4B). This effect is very pronounced in roots, where the adenylate pool is almost running out, and the capability to produce ATP becomes less. A similar effect
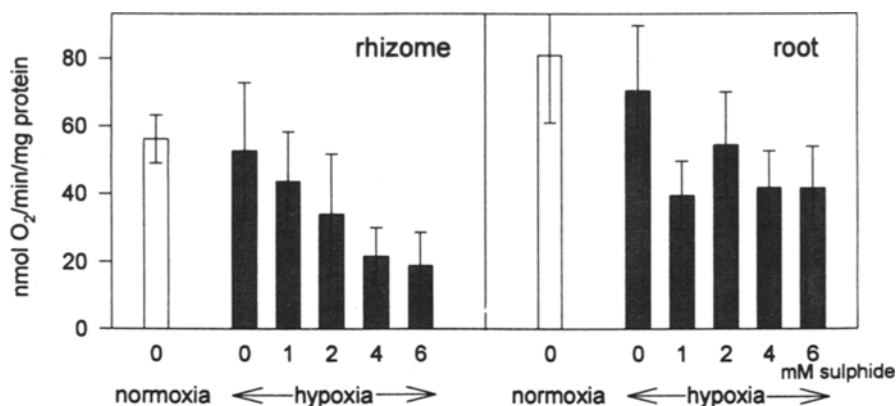
Fig. 5. Oxygen consumption following 24 h normoxia or hypoxia with increasing sulphide concentrations (0;1-6 mM). Mean of 5 independent experiments ± standard deviation.

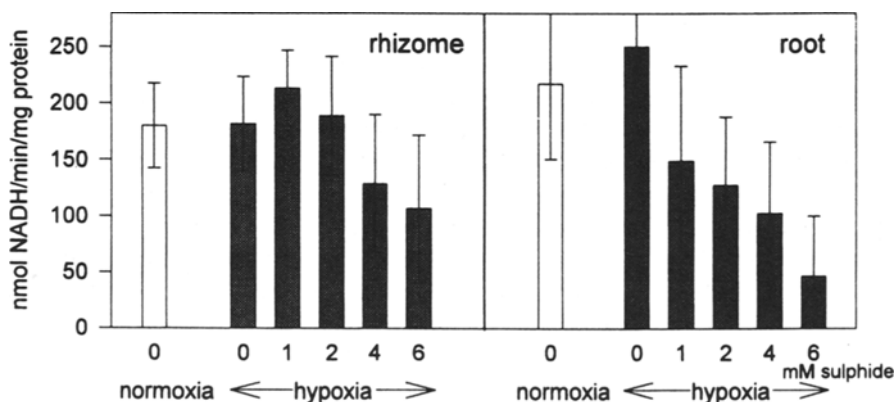

Fig. 6. Alcohol dehydrogenase activity after 24 h normoxia or hypoxia with increasing sulphide concentrations. Mean of 5 independent experiments ± standard deviation.

is observed in roots treated with only 1 mM sulphide for 4 d (Tab. 1). Obviously, the adenylate metabolism of roots responds more sensitively to sulphide than that of rhizomes.

Root segments and rhizome discs, immediately cut after sulphide treatment, can be used to estimate the remaining respiration capacity. A decrease compared to controls signifies a damage of mitochondrial functioning. Both tissues indeed show a decline with increasing sulphide concentrations (Fig. 5).

Compared to dryland species, most tissues of wetland species show very little change of ADH (= alcohol dehydrogenase) activity under oxygen deprivation (CRAWFORD 1992). Usually, ADH activities are several times above the needs to maintain anaerobic metabolism, and pyruvate decarboxylase (PDC) becomes the limiting step in ethanolic fermentation (BUCHER & KUHLEMEIER 1993). Furthermore, many other stresses increase ADH activities.

Table 2. Effect of sulphide on alcohol dehydrogenase activity in extracts of roots and rhizomes following 2 1/2 h of sulphide pre-treatment at 4 °C. Sulphide was applied as Na$_2$S in 0.1 M NaOH, without exceeding the buffer capacity. The controls were treated with NaOH only. Mean of 2 independent experiments.

Sulphide concentration	ADH activity (nmol NADH/min/mg protein)		% activity	
	rhizome	root	rhizome	root
0	245	288	100	100
0.5 mM	198	265	81	92
2.0 mM	95	168	39	58

Nevertheless, a substantial decrease of ADH activity could indicate an inhibited anaerobic ATP generation. As expected, hypoxia shows no or very little increase of ADH activity, but a strong decrease occurs under enhanced sulphide concentrations (Fig. 6). The activity loss of roots may be as high as six-fold. Prolonged treatment of roots at 1 mM sulphide leads to similar effects as do high concentrations (Fig. 7). Rhizomes are less sensitive. ADH is a metalloenzyme with Zn at its active site. Therefore, it is very probable that sulphide quickly inactivates the enzyme by simple interaction with the metal ion, as suggested by the results in Tab. 2. Incubation of root or rhizome extracts with up to 2 mM sulphide for 150 min at 4 °C lead to a significant decrease of ADH activity compared to the untreated extracts.

DISCUSSION

Sulphide toxicity is known for wetland plants, although they are adapted to anaerobic sediments and live in potentially sulphide containing soils (KOCH et al. 1990, PEARSON & HAVILL 1988). If the roots are not sufficiently protected by oxygen release (ARMSTRONG & ARMSTRONG 1988, ARMSTRONG et al. 1994), plant survival could be significantly affected.

Roots and rhizomes of common reed contain about 0.2 mM sulphide on a tissue water basis, if the environmental concentration lies at 1 mM sulphide. o-acetylserine may act as a sulphide acceptor to form cysteine that is converted to γ-glutamylcysteine and further on to the tripeptide glutathione (= γ-glutamylcysteinylglycine). GSH is usually the most abundant thiol. It accumulates mainly in rhizomes, much less in roots. GSH is non-toxic, and can be stored and transported easily. Furthermore, it serves as a reduced sulphur source, and may be included in several antioxidative defence systems (RENNENBERG & LAMOUREUX 1990). Usually, GSH is synthesized in leaves. However, the underground organs of reed plants are also able to form GSH from sulphide when shoots have been detached (data not shown). GSH levels do not exceed 300 nmol/g fw in rhizomes, and are below 60 nmol/g fw in roots, independent of the sulphide concentration applied, probably because of restricted glycine availability. Concentrations of more than 1 mM are rare in the interstitial water of freshwater sediments, but they are common in reed stands at brackish water sites (ARMSTRONG et al. 1996). However, even concentrations lower than 1 mM may be injurious because of the accumulative effect and the restricted thiol storage capacity of the tissues.

Sulphide clearly reduces ATP formation, as shown by the adverse effects on energy metabolism. Alcohol dehydrogenase activity is immediately reduced in the presence of sulphide, at least partially by Zn sulphide formation. Moreover, the post-hypoxic cytochrome pathway is inhibited. The remaining electron flow may be directed towards the alternative pathway which does not generate any ATP after the branching point. Nevertheless, roots and
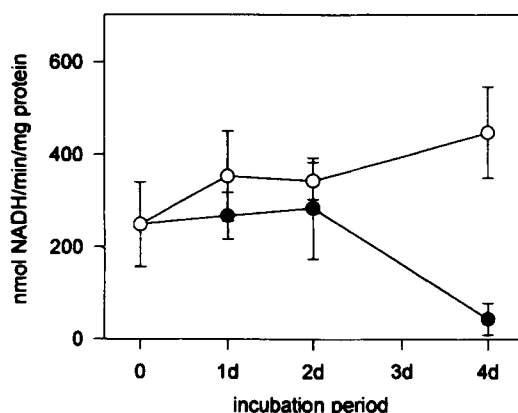
Fig. 7. Time dependent change of alcohol dehydrogenase activity in roots under hypoxia (O–O) or hypoxia + 1 mM sulphide (●—●). Mean of 3 independent experiments ± standard deviation.

rhizomes tend to keep adenylate energy charge high as long as possible, indicating an equilibrated metabolism of ATP producing and ATP consuming processes (PRADET & RAYMOND 1983). However, overall metabolic activity is continually decreasing because of the loss of adenylates with increasing time and sulphide concentrations. Under such conditions, tissues will gradually lose their viability (SIEBER & BRÄNDLE 1991). It is not known yet why reed roots are more sensitive to these processes than rhizomes. Common reed is not among the very intolerant plants, but it is much less tolerant than Acorus calamus L. (WEBER & BRÄNDLE 1996).

From our results, we conclude that underground organs of common reed (Phragmites australis) are severely sulphide stressed, and that the plants may die under the following conditions: (a) The sulphide exposed underground organs are anoxic or severely hypoxic, for example if the rhizomes are disconnected from atmospheric oxygen supply by the culms. (b) The sulphide concentrations of about 1 mM or less are replenished continually at the root surface and therefore overstretch the detoxification mechanisms. (c) Short-term presence of concentrations above 4-6 mM sulphide, e.g. local poisoning, is potentially lethal for non-aerated roots.

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