IRON SULPHIDE ACCUMULATION IN THE RHIZOSPHERE OF WETLAND RICE (Oryza sativa L.) AS THE RESULT OF MICROBIAL ACTIVITIES

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ABSTRACT

Studies on physiological disorders of wetland rice due to ferrous iron and sulphide toxicities revealed frequent accumulations of iron monosulphides (FeS \times H₂O in the rhizosphere. The processes involved in the formation of FeS are mainly bacterial iron-and-sulphate reductions. The significance of processes, which are both microbially catalyzed, is discussed in relation to physicochemical and biological soil parameters. The relevance of responsible bacteria in the rhizosphere and on the rhizoplane and their interactions with the rice plant are outlined.

Apparently, a lack of oxidation of these microsites by the rice roots is associated with this process of FeSaccumulation. Such an insufficient rhizospheric deration occurs frequently and is associated with nutrient-deficient or senescing plants or with plants heavily stressed, for instance, by transplantation. Under these peculiar conditions, the root death often occurs and, in certain cases, is preceded by a strong ferrous iron intoxication of the rice. An intensified sulphase reduction in the immediate rhizosphere of dead roots finally leads to a typical "post mortem" accumulation of FeS on the rhizoplane. However, in some cases such as spermosphere or young roots without aerenchym, a direct toxicity of sulphides may result in the death of germinating seeds and seedlings, accompanied by FeS precipitation.

Keywords: iron sulphide, waterlogged soils, rice physiological diseases, rhizosphere, anaerobiosis, sulphatereducing bacteria, ferric-iron-reducing bacteria

INTRODUCTION

The ability of wetland rice to survive in flooded soils is mainly due to their aerenchymatic air transport, which is then released into the rhizosphere. This oxidative protection may fail, however, resulting in ferrous iron and/or sulphide toxicities (Baba, 1958; Tanaka et al., 1968; Tanaka and Yoshida, 1970; Hollis et al., 1975; Jacq, 1977; Ottow et al., 1982, 1983). In certain soil conditions, the aeration mechanism is obviously ineffective. The accumulation of black ferrous sulphides in the rhizosphere and on the root surface is a common feature in all cases where ferrous iron and/or sul-

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phide toxicities are involved, but it may also be observed in the root zone of young and well-growing plants. In this paper, the nutritional status of rice, the bacterial processes responsible for the formation of ferrous sulphides in the rhizospheric soil and their effects on plant growth are discussed.

MATERIAL AND METHODS

Field surveys results, characterizations of both susceptible soils and sensitive rice cultivars (established during pots and microplots assays or field experiments in Senegalese agronomic stations), *in situ* physicochemical measurements (as pH and Eh determinations, or Fe(II) and sulphide contents in soil solutions and rhizospheric samples), laboratory analysis on leaves and soils, microbial enumerations (sulphatereducing and ferric-iron-reducing bacteria) and isolation technics of strains, were extensively described by Prade (1987).

The main results were obtained in Senegalese paddy soils originating from alluvial deposits and mangrove areas, situated either in the Senegal River Delta

TABLE 1

Sequence of microbial reductive transformations in flooded soils and theoretical equilibrium potentials of half-reactions at pH = 7 (from Patrick, 1960 and Yoshida, 1976; adapted by Ottow and Fabig, 1985)

	Measured redox potential (mv)				
Processes	transformations*	level	involved	E'0 (mv)**	
Respiration	>+400	>26	$O_{2}+4H^{+}+4e-\Leftrightarrow 2H_{2}O$	+814	
NO ₃ - respiration	+500 to +200	29-19	$2NO_{3} + 12H^{+} + 10e^{-} \Leftrightarrow N_{2} + 6H_{2}O$	+741	
Formation of Mn(I	I) +400 to +200	26-19	$MnO_{2}^{+}+4H^{+}+e- \Leftrightarrow Mn(II)^{+}2H_{2}O$	+410	
Fe(II) production	+400 to +180	26-18	$Fe(OH)_3 + 3H^+ + e - \Leftrightarrow Fe(II) + 3H_2O$	-185	
S ²⁻ production	+100 to -200	16–5	$SO_4 = +10H + +8e - \Leftrightarrow H_2S + 4H_2O$	-214	
CH_4 production	–150 to –280	7 - 2	$\dot{CO}_2 + 8H^+ + 8e^- \Leftrightarrow CH_4 + 2H_2O$	-244	

*Redox potentials (Eh) are comparable only if corrected for pH.

**E'o = Standard electrode potential at 50% of reduction at a pH of 7.0.

in the north of Senegal, or in the estuaries of the Casamance and Gambia Rivers in the south. These soils are irrigated and planted in flooded rice cultivars (directly sown or transplanted). Although they differ in some pedological characteristics (mainly in forms of clay), they may be considered as sulphate acid or potentially acid sulphate soils. They are often very clayey, quite saline and also characterized by a low pH (from 4.0 to 6.0 on dry soil samples at the end of the dry season).

RESULTS

Sequences of microbial transformations in flooded soils

The reduction processes in flooded soils proceed successively according to a well-established sequence which is determined by the standard electrode potentials of the redox equilibria involved, as previously studied by different authors, for instance, Patrick (1960), Yoshida (1976) or Ottow and Fabig (1985), and reported in Table 1. All reduction steps are mainly mediated by bacteria. The

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electron acceptors listed are increasingly electromotively sluggish and a relatively high "over-voltage" is required to shift the equilibria towards the reduced side.

Under sterile conditions. Fe(III)-oxides and sulphates are only slightly reduced. even at reduced conditions (Berthelin and Kogblevi, 1984; Berthelin and Boymond, 1978; Ottow, 1982). But ferric-iron reducers as well as sulphate-reducing bacteria, may use these compounds as electron acceptors during energy conservative metabolisms. The utilization of external oxidants during the microbial mineralization of organic substrates, permits a more efficient energy conservation (ATP-synthesis) than the fermentative processes. With increasing amounts of easily-decomposable organic matter in soils, the demand for electron acceptors certainly increases and, consequently, the sequential reduction of available electron acceptors (as presented in the ecophysiological sequence of Table 1) will be stimulated. Since nitrate and Mn(IV)-oxides are reduced soon after flooding, the main redox-buffering capacity is often provided by Fe(III)-reducible compounds and sulphates, which appear as the quantitative predominant electron acceptors (Inubushi et al., 1984) during the mineralisation of the soil organic matter (as presented in Fig. 1). However, sulphate-reduction in the bulk soil is prevented as long as Fe(III)reducible forms are available (Table 1). H₂S-formation will occur only in those soil compartments where reducible ferricoxides are exhausted. This is due to both higher energy of activation and lower redox level necessary for the sulphate-reduction, as well as to the specific metabolites (Fig. 1) required by the various sulphate-reducing bacteria. As opposed to iron-reducing bacteria, sulphate reducers require simultaneously — for their dissimilatory metabolism — strict anaerobiosis and presence of metabolites, either exudated by the plant roots or liberated during some anaerobic respirations such as denitrification, manganese-and-iron reductions or by fermentative process (Table 2). Significant amounts of ferrous iron are needed to precipitate the toxic H_2S produced (Jacq et al., 1986). Oxidation of organic substrates via ferric iron-and-sulphate reductions is demonstrated by eqns. (1) and (2):

$$CH_{2}O + 4Fe(OH)_{3} + 8H^{+} \rightarrow 4Fe^{++} + CO_{2} + 11H_{2}O$$
(1)

$$ADP + Pi \quad ATP$$

$$CH_{3}COO^{-} + SO_{4}^{+} + 3H^{+} \rightarrow H_{2}S + 2CO_{2} + 2H_{2}O$$
(2)

$$ADP + Pi \quad ATP$$
(3)

For details, the reader is referred to further literature (such as Münch and Ottow, 1980, 1982, 1983; Jacq, 1973, 1975, 1977; Freney et al., 1982).

Occurrence of iron-and-sulphate reductions in the rice rhizosphere and their relation to plant growth

The occurrence of iron-and-sulphate reducing bacteria in the rhizosphere of wetland rice is well documented (Jacq, 1973, 1975, 1980; Prade, 1987; Watanabe and Furusaka, 1980). Amongst the iron-reducing bacteria present on the rhizoplane of wetland rice in Senegal, *Enterobacter* spp. seem to be the dominant forms (19 strains among 56 isolates), but members of the genus *Bacillus* (22 strains, mainly *B. polymyxa* and *B. macerans*) and some *Pseudomonas* (8 strains) or *Clostridia* (7 strains) were also evidenced (Prade, 1987).

Table 2 lists the main sulphate-reducing bacteria (S.R.B.) evidenced in the spermo-

TABLE 2

Sulphate-(S.R.B.) and sulphur-(S°.R.B.) reducing bacteria and associated *Clostridium* in spermospherical and rhizospherical samples of Senegalese paddy soils (adapted from Freney et al., 1982, and Cord-Ruwisch et al., 1987; classification of Widdel, 1988)

Genus	Species and/	Occurrence and	Cell	Group	Main oxidized
	or strains	localization (1)	Form (2)	(3)	substrates
S.R.B.					
Desulfovib r io	S6 (4)	+,D,s,r,w	а	1.1	lactate
	S8 (4)	2+,D,s,r,w	a & b	1.1	lactate
	F2Y (5)	2+,SN,s,r,w	а	1.1	lactate
	'F2W (5)	2+,SN,s,r,w	а	1.1	lactate
	F3 (5)	3+,SN,s,r,w	а	1.1	lactate
	F0 (5)	3+,SN,s,r,w	а	1.1	lactate
	F2X (5)	2+,SN,s,r,w	а	1.1	lactate
	F1 (5)	3+,SN,s,r,w	а	1.1	pyruvate
	G1 (5)	2+,C,s,r,w	a&b	1.1	lactate
	K (6)	2+,D,r,w	а	1.1	lactate fructose
	A2 (7)	+,D,r,w	b & c	1.2	V.F.A.*
	A1 (7)	+,D,r,w	a & b	1.2	V.F.A.*
	B (7)	(+),D,r	а	1.2	lactate(?), V.F.A.*
Desulfotomaculum					
acetoxidans	sp. (8)	(+),D,s	d	1.2	acetate
Strain	N (9)	3+,SN,s,r,w	е	2	V.F.A.*
					L.C.F.A.**(C16)
Unidentified strain	GP (10)	+,SN,r	f	1.2 or 2	propionate
Desulfobulbus	sp.	(+),SN,r	g	1.2	lactate V.F.A.*(C3)
Desulfobacter	sp.	(+),SN,r	\mathbf{h}	2	acetate
Desulfosarcina	sp.	(+),SN,r	i	2	lactate L.C.F.A.**(C14)
Desulfonema	sp.	(+),SN,r	j	2	V.F.A.* L.C.F.A.**(C10)
S°.R.B.					
<i>Desulfuromonas</i> Associated	acetoxidans	3+,SN,s,r	b		acetate
Clostridium	aceticum "3T"	3+,SN,s,r,w	d & k		fructose, pyruvate

(1) Occurrence (in % of sample). 3+: >90%; 2+: >50%; +: >10%; (+): <1%.

Localization. D = Sengal River delta soils (north Senegal); SN - any rice field in Senegal; C = Casamance River estuary soils (south Senegal); s = spermospherical sample; r = rhizospherica sample; w = irrigation water sample.

(2) Cell Form. a: vibrio; b: vibrioid or short rod; c: curved rod; d: slightly curved rod, central round spore; e: sporulating rod, terminal spore; f: oval cell, round spore; g: oval or lemon shape; h: large oval rod; i: cells in packets; j: filamentous cell; k: very long rod, on fructose only.

(3) Group (... of S.R.B., according to their metabolism). 1.1: Incomplete oxidation of electron donors (end products: acetate + HCO₃-); acetate, others V.F.A.* and L.C.F.A.** not oxidized. 1.2: Incomplete oxidation, but V.F.A.* and some L.C.F.A.** as electron donors. 2: Complete oxidation (end product: HCO₃-) of any electron donor including V.F.A.*, L.C.F.A.** and some aromatic acids.

sphere and the rhizosphere of wetland rices in Senegalese fields (on 2,000 observed samples). The seven predominant strains (F0 to G1) were recently isolated. They belong clearly to the genus Desulfovibrio (group 1.1). Some studies on their physiology and carbon nutrition (electron donors and fermentative substrates) established clearly that they may be considered as new strains, and that they are mainly provided with suitable carbon substrates (lactate, pyruvate, and even some sugars, such as fructose) by roots exudates. This latter result may explain why they are located very close to the root-soil interface. In 95% of spermospherical or rhizospherical treated samples, two to four of them were evidenced. The isolated Desulfovibrio differ, by at least one and generally two or three significant properties, from similar Desulfovibrio so far encountered in tropical areas. It was also ascertained that their population levels in rhizospheric samples $(about 10^7 to 10^9 cells per g)$ are significantly higher than those of the sporulating forms of S.R.B.

Three Desulfotomaculum strains (group 1.1 or 2) were evidenced. They can also utilize, as carbon substrates, some low molecular organic compounds accumulated during the fermentative process (as reported in Fig. 1). Sporulating forms were observed only in 60% (N) or in 5% (GP) of samples, but they remain significantly dominant in the rice root zone of decaying plants. Before our study, such Desulfotomaculum strains were not evidence in soils, and the presence of Desulfotomaculum acetoxidans remains uncertain. The N strain may not survive in pure culture because it is always strongly associated with a Clostridium (the "3T strain", identified as C. aceticum; it does not correspond to any classified strain. It was, therefore, suggested that the dominant strains of sulphate reducing bacteria evidence in Senegalese rice fields were specific and quite unknown. Further taxonomic studies are needed to ascertain whether their peculiar characteristics may be sufficient to define new species, but such properties partly explain their adaptation to ecological conditions encountered in the rice field biotope.

- (9) N strain was isolated 3 times, but it may not survive on pure culture when separated from the associated 3T strain (*C. aceticum*); some properties are quite similar to those of *Desulfotomaculum orientis* (group 1.2), but this strain differs from *D. orientis* because it can use some L.C.F.A.** (as group 2 strains).
- (10) GP's cell forms are typical of those of *Desulfobulbus propionicus* (group 1.2) but their growth on propionate is very slow (doubling time: 3-4 months); this strain sporulates.

*V.F.A. = Volatile Fatty Acid; **L.C.F.A. = Long Chain Fatty Acid.

TABLE 2 (Cont'd)

Notes on Strain Specificities.

⁽⁴⁾ S6 and S8 strains are quite similar to Desulfovibrio desulfuricans or vulgaris.

⁽⁵⁾ Isolated, but different from any described Desulfovibrio

⁽⁶⁾ Isolated, unidentified: K strain differs from *Desulfovibrio fructosovorans*, even if it may grow on fructose as electron donor (but only when yeast extract is added)

⁽⁷⁾ Not isolated strains, strongly associated with 3T strain (*C. aceticum*); as for *Desulfovibrio baarsii*, no growth on lactate.

⁽⁸⁾ This strain differs from the typical strain described by Widdel and Pfennig (1977): N. Pfennig, personal communication.

HIGH MOLECULAR ORGANIC COMPOUNDS + 02



Fig. 1. Diagram of the sequential degradation of the organic matter in flooded soils (modified from Freney et al., 1982).

Most of the highly active iron-reducing bacteria are anaerobic or facultatively anaerobic, and thus, some of them can grow in aerobic compartments of the rhizosphere (Prade, 1987), where surplus oxygen is released by healthy roots. It appears that they are very efficient during the settlement of anaerobiosis. Sulphate-reducing bacteria, however, are strictly limited to the anoxic compartments, even if spores and non-sporulating cells may survive in oxic layers of soils (Furusaka et al., this volume).

The population dynamics of both microbial groups (iron- and sulphate-reducing bacteria) on the rhizoplane of rice, are shown respectively in Figs. 2 and 3. It was useful, for enumerating S.R.B. (as reported in Fig. 3) to test three different electron donors. The S.R.B. enumerated on the lactate medium (Fig. 3a) are those pertaining to group 1.1 (typical Desulfovibrio: lactate or pyruvate as preferential oxidized compound). Their relative numbers are about 1,000 times higher than simultaneous numbers of S.R.B. from groups 1.2 and 2 (mostly Desulfotomaculum or related strains, and perhaps some other Desulfovibrio) which may oxidize acetate (Fig. 3b) accumulated by the previous group of S.R.B., or resulting, as intermediary product, from the organic matter degradation. They are about 10,000 times higher in number than the long-chain-fatty-acids-oxidizing S.R.B. (Fig. 3c). The observed cells on this latter electron donor are only those of N strain, morphologically identical to those of Desulfotomaculum orientis and nutritionally similar, except for their slow growth rate on palmitate (a C-16 L.C.F.A.), unknown for this last species.

Apparently, sulphate- and iron-reducing bacteria greatly increase in number at the beginning of the reproductive growth phase of rice. In the case of iron-reducing bacteria, this increase can probably be ascribed to an enhanced exudation of carbohydrates and other metabolites during heading of rice panicles. Deficiencies in P, K, Ca and Zn may enlarge the root permeability, that enhances exudation, oxygen-consumption and iron-reduction, finally leading to severe iron toxicity (Benckiser et al., 1984a and b; Jacq et al., 1986; Prade et al., 1988). In fact, a disappearance of red iron oxides on the root surface during flowering has been frequently observed on such iron-toxic soils, proving that iron gets reduced (Kimura et al., 1982; Prade, 1987).

The significant increase of populations of all observed forms of sulphate-reducing bacteria (including sporulating cells). during the reproductive stage of rice may be explained by an increased rate of senescing or dead roots, occurring naturally as the result of excessive flow of iron into the rice plant (Jacq et al., 1986; Prade, 1987). In non iron-toxic soils (as in well-fertilized rice fields of the Senegal Delta Region), the increase of sulphate reducers at flowering is accompanied by a significant decrease of redox potential (Lower et al., 1982). That may be due to an insufficient oxidative power of the senescing roots, the rhizosphere of which became rapidly strongly anaerobic in such waterlogged soils.

Usually, the amounts of ferrous iron and sulphides in the soil solution of flooded soils are significantly higher in planted than in unplanted soil (Figs. 4 and 5). This result shows that, despite the aeration mechanism of the roots, iron-reduction processes in the bulk soil are stimulated by the physiological activity and by the growth of rice roots. Contrary to the rhizospherical stimulation of iron-reduction — which probably takes place during the whole



Fig. 2. Populations of ferric iron-reducing bacteria (C.F.U./g dry root) in the rhizoplane of a wetland rice (IR8 cultivar) during the growth cycle in a microplot experiment in Senegal (Prade, 1987).

growth cycle — sulphate-reduction occurs mainly either in the spermosphere (where the oxygen release is obligately absent) and/ or in the rhizosphere, where the aerenchymatic oxygen release is still weak, such as during tillage, and mainly from flowering to harvest and, above all, during the senescence of the root system (Loyer et al., 1982). Such observations correspond to the population dynamics of the sulphate-reducing bacteria in the root zone (Fig. 5).

Numbers and activities of iron-reducing bacteria seem to be enhanced by nutrient deficiencies of the rice plant (Figs. 2, 4 and 6), but this was not observed so significantly for sulphate reducers (Fig. 3). Rice roots, particularly those suffering from the "secondary" iron toxicity during flowering (as a consequence of P and K deficiencies) are often blackened by ferrous sulphide precipitations (Jacq et al., 1987). Since abundant sulphate reduction on the root system is expected only ir iron oxides have been completely reduced, it can be suggested in these cases that roots exposed to high sulphide levels may have been damaged earlier by high ferrous iron concentrations. In most cases, when high sulphide levels and increases in sulphate-reducing bacteria numbers in the rice rhizosphere



Fig. 3. Populations of sulphate-reducing bacteria (S.R.B.) in the rhizoplane of a wetland rice (IR8 cultivar) during the growth cycle in a microplot experiment in Senegal, enumerated on three different electron donors: (a) lactate; (b) acetate; (c) palmitate (Prade, 1987).

were observed, the root death had occurred previously or simultaneously (Fig. 7) as a result of the ferrous iron toxicity and then, perhaps, of other stresses or even natural senescence. Apparently, the accumulation of ferrous sulphides on the roots of wetland rice should be considered as the result, rather than the cause, of root death. This is



Fig. 4. Fe(II)-concentrations (ppm) in the rhizospheric soil solution of differently fertilized wetland rice (IR8 cultivar) in comparison with an unplanted soil during a field experiment in Djibelor/Senegal (Prade, 1987).

also indicated by the observation that in iron-toxic soils, rice roots penetrate easily through soil horizons blackened by ferrous iron sulphides if their oxidative power is functioning properly (Jacq et al., 1987).

The rapid accumulation of sulphides in the spermosphere (as shown in Fig. 5) suggests that iron-reduction had been rapidly completed, due to the relatively high availability of seed-borne carbohydrates liberated during germination. Similarly, in the rhizosphere of freshly transplanted rice seedlings, reduction processes can be stimulated by the root damage and by the low aerenchym formation in the primary roots. In these cases, direct sulphide toxicity can't be ruled out since the germinating rice seeds as well as young seedlings are lacking an oxidative mechanisms which is only established later by the formation of new aerenchymatic roots. This fact may explain, at least, the first manifestation of rhizospherical sulphide toxicity from the end of germination to tilling (Fig. 5). Such sulphide toxic effects on young seedlings (when they were directly sown) may occur during the same growth period of plants suffering from the "primary" ferrous iron toxicity, more specific to transplanted rice.

Different ways of formation of ferrous sulphides in the root zone are summarized using the four theoretical diagrams reported in Fig. 8. The extent of the FeS deposit



Fig. 5. Concentrations of total sulphides (ppm) in rhizospheric and non-rhizospheric samples of rice (IR8 cultivar) during a microplot experiment (Jacq, 1980).

(represented by "spotwise FeS" may easily be evidenced by its very dark colour (very different from the usual yellow to brown colours of the healthy roots) and its accumulates differently according to the age and/or the nutritional status of the rice plant.

The precipitation of Fe(III)-oxides on the health roots (i.e., from well-fertilized plants placed under moderate flooding conditions), which are free from any sulphides or ferric iron disease (in spite of the appearance of a slight spot of ferrous-iron sulphide), is explained using Fig. 8b. As long as P- and Kuptake by roots remains effective and roots are not damaged, the oxygen flow from aerial parts of the rice plant is sufficient to oxidize small amounts of Fe(II), and Fe(III)oxides may precipitate on the root-soil interface (rhizoplane). Similarly, in the rhizosphere, FeS (also microbially-produced at a low level) may be oxidized, either by the oxygen flow from roots and/or by microbial processes (for instance, by *Thiobacillus denitrificans*: Jacq and Roger, 1978). It may accumulate only beyond the root-depending microsites where these oxidative processes occur, quite far from the rhizoplane: any soluble form of sulphide is available to poison the rice that remains healthy.

Young roots in quickly waterlogged soils (Fig. 8a) as well as the older roots of senescing plants suffering from nutrient deficiencies (Fig. 8c) are very sensitive to both sulphides and ferrous iron, even if they do not appear, when toxic levels are reached, so darkened that decaying or dead roots fre-



Fig. 6. Effect of an equilibrated fertilization (N+P+K+Ca+Mg) on the populations of ferric-iron-reducing bacteria (expressed as percent of the total population of aerobes) on the rhizoplane of IR8 rice, in microplot experiments during two consecutive seasons (Prade, 1987). Significant differences are indicated by *.

quently observed after flowering (Fig. 8d). Because they often in a few days, a black deposit of the unstable ferrous iron monosulphide may be rapidly observed on the rhizospheric soil, providing the root sampling is done under reducing conditions. At the end of germination (Fig. 8a), the aerenchym of young roots is not yet established, resulting in an ineffective oxygen-flow into the nearest rhizosphere. But the important carbon substrates exudation simultaneously stimulates the sulphate reducers and the iron-reducing microflora. Therefore, Fe(III) reduction mainly occurs close to the rhizoplane and the resulting FeS accumulates at a very short distance from the root surface.

Similarly, if the young root system was damaged during transplantation or, more frequently, when insufficient P- and Klevels are reached (Fig. 8c), in spite of a normal oxygen flow from rice aerenchym, dissolution and reduction of Fe(III) oxides occurs on the rhizoplane, resulting in a significative FeS accumulation — generally low, but sometimes quite high, depending on amounts of soluble sulphides produced by sulphate reducers.

When the rice plant is dead (Fig. 8d), the oxygen-exudation into the rhizosphere is not, of course, possible and the whole rhizospheric soil becomes strongly anaerobic. The root decomposition provides the sul-



Fig. 7. Production of total sulphides in the spermosphere (A) or in the rhizosphere of young (B) and old (C) plants in relation to the oxidation status of the microsite [indicated by $\log_{10}(S.R.B./S.O.B.)^*$] and to plant health (Jacq and Roger, 1978). Living and dead plants are designated by Δ and \blacksquare respectively; *S.R.B. = lactate-using Sulphate-Reducing Bacteria; S.O.B. = Sulphate-Oxidixing Bacteria (mainly *Thiobacillus denitrificans*).

phate reducers with high quantities of useful carbon-substrates, allowing a very intensive *post mortem* sulphide production. These sulphides immediately react with free Fe(II) forms which do not enter the root and an important amount remains subsequently available in the whole rhizosphere: a typical widespread FeS deposit may be observed all around the dead root system.

CONCLUSIONS

Microbial ferric-iron and sulphate reductions may occur at a relatively high rate in the rhizosphere of wetland rice, under moderate to severe flooded conditions. This can lead to an excessive uptake of Fe(II) and

Fig. 8 (Overleaf). Schematic presentation of the solution concentrations (E.C.) of Fe(II) and sulphides (S=) in rhizosphere, and of the precipitation of ironminerals in the rhizoplane, of wetland rice (modified from Prade, 1987). (a) Young roots, without aerenchyme; (b) intact roots, with aerenchyme; (c) nutrientdeficiency or senescing roots; (d) dead roots.

-----: Fe(II) concentrations; -----: FeS concentrations; d.f.r. = distance from rhizoplane; E.C. = Equilibrium Concentration (depending from both pH and Eh); * level of Fe(II) entering the root; No scale = arbitrary scale; Spotwise: presence of FeS, evidenced by darkening.



to the precipitation of ferrous sulphides on root rhizoplane or in the root vicinity, when the rhizospheric aerate mechanism of rice is not working effectively. Iron and sulphate reductions depend largely on the availability of organic substrates, which explains their preferential occurrence in the rice spermosphere and rhizosphere. The usually observed post mortem accumulation of ferrous sulphides in the close rhizosphere may reflect an increase in organic substrates after the death of injured roots. Thus, the appearance of ferrous sulphides in the rhizosphere can only serve as an indicator of root damage, senescence or death, but should not be explained as the result of *direct* sulphide toxicity. It can be encountered in plants suffering from iron toxicity or from other soilderived stresses which are associated with insufficient aeration power of the root. However, when ferrous iron sulphides appear around germinating seeds - which generally involves death — we may assume that the main microbes responsible are sulphate reducers, even in the spermospherical sulphate reduction is obligately to be preceded by a complete reduction of reducible Fe(III) in the vicinity of germinating seeds.

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